

1994

# The effects of calcium chloride, sodium chloride, and tripolyphosphate injections on the tenderness, water holding capacity and microscopic structure of beef muscle

Dirk Douglas Beekman  
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**The effects of calcium chloride, sodium chloride, and  
tripolyphosphate injections on the tenderness, water holding  
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**Beekman, Dirk Douglas, Ph.D.**

**Iowa State University, 1994**

**U·M·I**

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Ann Arbor, MI 48106**



The effects of calcium chloride, sodium chloride, and  
tripolyphosphate injections on the tenderness, water holding  
capacity and microscopic structure of beef muscle

by

Dirk Douglas Beekman

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

Department: Animal Science  
Food Science and Human Nutrition  
Major: Meat Science

Approved

Signature was redacted for privacy.

In Charge of Major Work

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For the Graduate College

Iowa State University  
Ames, Iowa

1994

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## GENERAL INTRODUCTION

The need to create a beef product of uniform tenderness has recently become a top priority for producers and processors due to consumer driven demands. The reason one animal produces tender steaks while another produces tough steaks has perplexed and challenged meat scientists and the meat industry for many years. Tenderness is known to be affected by a large number of independent variables. Because of the effects of ante and postmortem factors, the meat/muscle system is one of the most complex systems in nature to study. The effect of postmortem age on the myofibrillar proteins is of particular interest. Consequently, achieving uniform tenderness is a challenging problem requiring creative and innovative approaches.

Many methods have been studied in an attempt to enhance beef tenderness and improve water holding capacity (WHC). Postmortem aging improves tenderness (Huff-Lonergan et al, 1994b) and these improvements in tenderness have been shown to occur in the myofibrillar proteins (Olson et al., 1976; Huff-Lonergan et al., 1994b). Recently, injection of beef loins with solutions of calcium chloride ( $\text{CaCl}_2$ ) has resulted in rapid tenderizing effects (Wheeler et al., 1991; Wheeler et al., 1993). Addition of  $\text{CaCl}_2$  to the meat system has allowed a significant increase in the tenderness of treated meat samples, possibly due to the actions of the calpain enzyme system on myofibrillar proteins including titin and nebulin. However, at certain concentrations of  $\text{CaCl}_2$  some off-flavoring of the meat products may

be noted, and some researchers have questioned the effectiveness of  $\text{CaCl}_2$  injection treatments (Benito-Delgado et al., 1994). Because approximately 20% of the population can detect the bitterness sensation, when conducting taste panels it becomes very important to note which, if any, panelists are sensitive to bitterness sensations. It is likely these same 20% would possibly reject a  $\text{CaCl}_2$  injected product because of bitterness. NaCl and phosphate have also been shown to have an affect on the WHC of beef muscle, and consumers may also indicate an increase in tenderness in muscle injected with these compounds.

The effect of injecting compounds in muscle on certain proteins of the myofibril needs to be systemically studied in order to identify the effects on tenderness, as well as WHC and taste sensations. These changes could be detected using SDS-PAGE techniques. Solubilization and degradation of titin and nebulin after incubating myofibrils with salt and phosphate solutions and extraction of titin has been observed by researchers using SDS-PAGE (Paterson et al., 1988; Huff-Lonergan et al, 1994a). The use of gradient techniques of SDS-PAGE allows the detection of protein degradation over a large range of molecular weights. The detection of a 30,000 dalton component as a rapid determination of steak tenderness may be prove to be beneficial to the meat industry.

This study was designed to observe the effects of injecting steaks with NaCl, sodium tripolyphosphate, and calcium chloride dissolved in aqueous solutions on palatability, physical properties and



degradation of myofibrillar proteins. Specifically the objectives were to determine: 1) the effect of injecting calcium chloride, sodium chloride and tripolyphosphate in beef on the appearance of the 30,000 dalton component, 2) effects of the injection treatments on palatability, mainly tenderness, of *longissimus dorsi* steaks from A maturity carcasses, 3) effect of the injections on myofibrillar proteins using SDS-PAGE and indirect immunofluorescence technology, 4) effect on specific sensory aspects, including juiciness, bitterness, saltiness, and tenderness, of injected samples, and 5) the effect of the aforementioned injection treatments on the water holding capacity and myofibril fragmentation index of *longissimus dorsi* steaks.

#### Explanation of Dissertation Format

This dissertation is presented as a general introduction, a general review of literature, an individual paper and a concluding general summary. References cited in the general introduction and literature review follow the general summary. All citations of references are in accordance with the style manual used by the Journal of Food Science to which a portion of this paper will be submitted. The individual paper consists of an abstract, introduction, materials and methods, results and discussion section and a conclusion.

## GENERAL REVIEW OF LITERATURE

### Main Categories of Tenderness

Tenderness is a field of research which has been extensively studied, yet the mechanism(s) of the postmortem tenderness reaction remains a mystery. Consequently the investigation of tenderness is academically stimulating. One reason tenderness receives so much attention is its large economic impact to the consumer. If it was possible to determine why one steak is tough while another is tender, the economic ramifications of creating a uniformly tender product would be profound (Morgan et al., 1991b). There are essentially two main categories of tissue components which contribute to the tenderness of muscle; connective tissue/collagen and myofibrillar proteins. The connective tissue contribution to tenderness is commonly referred to as background toughness.

### Connective tissue aspects

Collagen comprises only 1-6% of muscle by weight (Bendall, 1967) and yet it has been directly involved in background meat toughness (Bailey, 1972), shrinkage during cooking (Light et al., 1985), and meat fracture when chewed (Purslow, 1985). So it would seem that it could possibly play a role in meat tenderness. In 1907 Lehmann discovered the toughening relationship between connective tissue and meat. Much research has been completed since then, and it is now believed that differences in the age, breed and sex of an animal will also have an effect on the stability of the collagen molecule (Cross et

al., 1973). Collagen in the meat system is a triple helix structure comprised of three polypeptide chains (Bailey, 1989). The triple helix then forms tropocollagen molecules lined up in a quarter stagger overlap and the tissue formed is classified as fibrous collagen. Non-fibrous, and filamentous collagens also exist, but does not appear to significantly affect tenderness (Bailey, 1989). It appears that the toughening of the fibrous collagen molecule over time occurs due to the formation of intramolecular covalent, or multivalent cross-links, and these cross-links then contribute most of the strength of collagen (Bailey, 1989). Several studies have suggested that the total amount of collagen present in muscle is not the major contributor to tenderness, but rather the amount of soluble collagen (Hill, 1966; Kruggel et al., 1970; McKeith et al., 1985). Hill in 1966 demonstrated that during cooking there was less collagen solubilized in old animal muscle as compared with young animal muscle. A number of studies have also demonstrated that the overall amounts of soluble collagen will decrease as the chronological age increases (Goll et al., 1964; Hill, 1966; Cross et al., 1973; Miller et al., 1987). Recent research of Stanton and Light (1990) suggests that the endomysium is the first connective tissue barrier attacked by lysosomal cathepsins once the sarcolemma has been breached, and this endomysial degradation is the main connective tissue component contributing to tenderness; however, these results have not been repeated by other researchers.

Some researchers presently believe that collagen is not an important factor in meat because once the meat is cooked the collagen

forms a gelatin and essentially melts (Davey and Gilbert, 1974a; Davey, 1983). Once the collagen is converted to a gelatin, nearly all of its strength is lost. A study by Seideman et. al. (1987), which used a large number of young beef as experimental units, found no significant relationship between total collagen and tenderness, or soluble collagen and tenderness. Most researchers now call the collagen effect "background toughness" which is a certain threshold level of tenderness due to the amount of collagen. For example the psoas major muscle has very little collagen, whereas the semimembranosus has much more collagen and is less tender (Ramsbottom et al., 1945). Overall it appears that collagen plays a role in toughness, when comparing cow meat to veal meat, however in young market animals it appears to be a less significant factor.

A second connective tissue named elastin has been found and studied in bovine muscle, but it has been shown not to be a critical component in beef tenderness (Cross et al., 1973).

### Myofibrillar tenderness

Myofibrillar proteins have been extensively studied with the objective of determining their relationship to tenderness. However, due to the complexity of skeletal muscle it is first necessary to understand the myofibrillar protein composition before relating any protein to tenderness. These proteins, which make up the myofibrils of muscle comprise over half of the total skeletal muscle proteins (Parrish and Lusby, 1983), and are the means for the contraction/relaxation reactions to create movement (Huxley, 1958;

Murray and Webber, 1974). Once researchers such as Huxley in 1958 questioned how the molecular machinery of muscle, or chemical energy was converted into physical movement and showed the thick and thin filament structure of muscle (including the interaction of the filaments), a large amount of research was begun in the muscle biology area in an effort to isolate the effect of contraction. Presently researchers are discovering new proteins believed to be involved in the contractile process, and the consequences of these proteins on meat tenderness.

### Myosin

Myosin was first isolated by Kuhne in 1859, and is the principal component of the contractile system, comprising approximately 45% of the myofibrillar proteins (Murakami and Uchida, 1985). The myosin molecule itself is a rod shaped protein with two globular regions at one end termed myosin heads. The myosin molecule can be classified to a greater degree by six protein chains which comprise the molecule. Two of the chains, the heavy chains, comprise the entire rod section and most of the myosin head regions. The other four chains, the light chains, comprise the remainder of the myosin head regions. As the names would suggest the myosin rod portion is an  $\alpha$ -helical coiled-coil structure, while the myosin heads are comprised of irregular folds making it a globular structure (Pette and Staron, 1990). In skeletal muscle it appears that myosin has three basic functions: it acts as an ATPase, it binds actin and it can form filaments. The ATPase and actin binding properties are located in the head regions, and the rods interact

to form filaments (Squire, 1986). The myosin molecule overlap each other forming a core with the head regions protruding outward, and are located in the A-band region of skeletal bovine myofibrils. These cores of myosin molecules including the attached head regions comprise the thick filaments. The myosin molecule can be cleaved naturally and/or chemically into a number of subunits including the head and/or the rod regions. Two compounds able to cleave the myosin molecule are papain and trypsin. Papain cleaves the myosin molecule into globular head regions (heavy meromyosin subfragment-1) and the rod portion. Trypsin cleaves the intact molecule into a partial rod portion (light meromyosin), and the head regions with a portion of the rod attached (heavy meromyosin). Trypsin will also cleave the entire papain cleaved rod portion into light meromyosin and a portion of the rod termed heavy meromyosin subfragment-2 (Szent-Gyorgyi, 1953; Pette and Staron, 1990).

### Actin

In the early 1940s actin was discovered by Straub (1942), and although he did not realize it at the time he had also discovered that the monomeric form of actin, G-actin, polymerizes into the filamentous form in nature termed F-actin. The F-actin filaments form a double helix to yield actin filaments. The actin filaments extend from the Z-line into the A-band area, where they essentially surround the thick filament. The pioneering work of Huxley (1958) explained a theory of muscle contraction which is still used to this day. In this theory myosin heads and actin are at times bonded at specific areas on the

actin molecule, and during contraction the I-band of myofibrils decrease in length while the A-band area (containing the actin, and myosin cross-bridges) remains constant. It was also noted that the actin filaments themselves did not change in length, so it was concluded that the filaments must slide past, or interdigitate with each other. Further work of Murray and Webber (1974) helped to clarify some of the unanswered areas of the Huxley model. Myosin and actin together are the two main contractile proteins sufficient for contraction. Murray and Webber (1974) explained how myosin, actin, as well as tropomyosin and troponin were involved in the thick and thin filaments interactive process to create contraction and relaxation. When rigor mortis begins, actin and myosin become tightly bound to each other creating a situation termed actomyosin toughness, which may contribute to the overall toughness of skeletal muscle. Trout and Schmidt (1983) noted a degradation of the actomyosin complex when NaCl was added to the meat system, and hypothesized that this could have an effect on muscle tenderness.

#### Tropomyosin and Troponin

Tropomyosin is a main protein (5% of myofibrillar proteins) in striated muscle which plays a key role in the regulation of the contractile process (Bailey, 1948; Ebashi and Endo, 1968). Tropomyosin lies on the thin filament in a head to tail array, and in conjunction with troponin serve to regulate the calcium induced interaction of myosin and actin to bring about the contraction of muscles. It is difficult to explain how tropomyosin functions without also explaining its

relationship with troponin. When calcium ions are released from the sarcoplasmic reticulum ( $\text{Ca}^{++}$  binding membrane in skeletal muscle) the ions are bound to a subunit of troponin, therefore acting as a regulatory protein. Troponin is made up of three subunits, C, I and T, and is in parallel alignment with the thin filament at regular intervals.

Troponin C acts as the calcium binding subunit, while the I subunit inhibits actomyosin ATPase activity. Troponin T is the subunit which binds to tropomyosin. This arrangement therefore creates an indirect link to the thin filament (Shaub and Perry, 1969; Zot and Potter, 1987). Following is a brief review of the contractile process involving the proteins mentioned: first  $\text{Ca}^{++}$  is accumulated in the sarcoplasmic reticulum (SR) against a  $\text{Ca}^{++}$  concentration gradient. The  $\text{Ca}^{++}$  diffuses inside the SR toward the cisternae (area approaching the t-tubules). At the end of the cisternae there are projections termed "feet" which is the channel the  $\text{Ca}^{++}$  passes through. The feet are also the ryanodine receptors which when stimulated with ryanodine serve to "lock" the channel in the open position if there is a low calcium concentration (less than 10 mM  $\text{Ca}^{++}$ ), or close the channel if there is a high  $\text{Ca}^{++}$  concentration (greater than 100 mM  $\text{Ca}^{++}$ ). When a stimulus arrives at the cell, it continues down the t-tubules causing the  $\text{Ca}^{++}$  channels to open which results in a rapid release of  $\text{Ca}^{++}$  to the intracellular area. Troponin C then binds the released  $\text{Ca}^{++}$  which creates a conformational shift in the troponin-C molecule. This shift in turn causes a contractile cycle to occur allowing troponin-I to bind to troponin-C. Troponin-T now is able to bind to troponin-I, and this in turn allows



tropomyosin to bind to troponin-T. Tropomyosin, because of these conformational shifts moves into the groove of the thin filament uncovering contractile sites on actin. Consequently these conformational changes allow an increase in the myosin cross-bridge numbers attaching to actin, an increase in actomyosin ATPase and ultimately an increase in the tension of the muscle (Zot and Potter, 1987).

#### Titin (Connectin)

The discovery of this protein is credited to Wang et.al (1979); however, it should be noted that at approximately the same time Maruyama (1976) was discovering a mixture of proteins they dubbed connectin which was later purified and found to be the same protein as titin (Maruyama et. al., 1981). Titin has been found to migrate in SDS-PAGE as a close doublet with the bands denoted T1 and T2, with T1 being of a higher molecular weight. Titin is a very large protein (2,800 kDa; Kurzban and Wang, 1988; 3,000 kDa Trinick, 1992) and represents about 10% of the myofibrillar protein. Does titin comprise a third set of contractile filaments? This has been a very controversial subject since the initial discovery of titin. It appears that it is structural, and may be involved in relaxation. Locker (1987) proposed a complex filament system which he named T-filaments, of which titin was an integral component. In Locker's theory, six T-filaments surrounded one A-band filament, and C-protein acted to connect two T-filaments with one A-filament. Another theory of a third contractile/elastic filament system is that of Maruyama (1985). In this model connectin "nets" are

linked to other elastic type nets (possible comprised of nebulin) which are in turn connected to a set of elastic filaments which are connected to the Z-line. This theory is somewhat similar to that of Wang et. al. (1984a). In this model, a third set of filaments comprised of titin and possibly another large molecular weight protein, nebulin, connect Z-line to Z-line. Titin, in this model, forms a strand around thick filaments, and fuses with nebulin at the N<sub>2</sub> line area, then continues on to the Z-line. Titin has been linked to tenderness by researchers who believe in a variety of titin filament models (Young et al., 1980; Lusby et al., 1983; Zeece et al., 1986; Paterson and Parrish, 1986; Paxhia and Parrish, 1988; Ringkob et al., 1988; Anderson and Parrish, 1989; Beekman and Ringkob, 1992). Titin has also been linked to water holding capacity (Paterson et al., 1988) and the growth and development (Parrish et al., 1988; Whiting et al., 1989) of skeletal muscle.

### Nebulin

This large myofibrillar protein (600 to 900 kDa, Hu et al., 1986; Locker and Wild, 1986; Wang and Wright, 1988; Jin and Wang, 1991) comprises 5% of the myofibrillar proteins, and was discovered by Wang et al. (1979) who termed it band three. As was mentioned earlier, nebulin seems to be in close approximation in the myofibril with titin. Early researchers believed that nebulin was bead like and comprised the difficult to isolate N<sub>2</sub> lines of the myofibril (Wang and Williamson, 1980; Wang, 1981; Robson and Huiatt, 1983; Locker, 1984). However, papers by Furst et. al. (1988), and Wang and Wright (1988) caused

researchers to alter their previous theories. These two papers made it appear that nebulin actually ran perpendicular to the  $N_2$  lines and is quite long (roughly one  $\mu\text{m}$ ), and runs adjacent to the thin filaments. The  $N_2$  line is now believed to have been an artifact of the sample preparation. Some researchers have speculated that nebulin may actually comprise a fourth set of filaments (Nave et al., 1990), or actually be an integral part of the thin filament (Robson et al., 1991). It appears that nebulin may be as important to tenderness as titin since it is rapidly degraded in the early postmortem period, and runs from the Z-line to the thin filaments in what appears to be a myofibril integrity apparatus (Robson et al., 1991).

#### Other proteins

There are several other myofibrillar and cytoskeletal proteins which have been the subject of a number of studies, and may be linked in some fashion to tenderness. Located in the interior portion of the Z-line are  $\alpha$ -actinin and Z-protein.  $\alpha$ -actinin is the major F-actin associated protein of the Z-band which can be used in experiments to determine the location of nebulin in the myofibril, and its attachment to the Z-line (Nave et al., 1990). The main purpose of this protein is to bind actin to the Z-line area (Ebashi and Ebashi, 1965; Maruyama and Ebashi, 1965). Z-protein is a lesser studied protein which has also been shown to be located in the Z-line and may be involved in anchoring proteins which enter the Z-line (Ohashi and Maruyama, 1989). A protein located on the exterior portion of the Z-line is desmin. Desmin (1-2% of myofibrillar protein) forms long, transverse filaments

approximately 10 nm in diameter, which are located at the periphery of the Z-line (Robson et al., 1984; Maruyama, 1985). It appears that one function of desmin is to aid in connecting myofibrils to one another and to the cell membrane (Robson et al., 1991). There are also several myofibrillar proteins which do not receive as much attention as the main contractile proteins, yet are still important in the overall workings of the myofibril. C-protein is a protein located in the myofibril at certain areas on the thick filament (Bennett et al., 1986). This protein has been located in each half of the A-band, labeling nine perpendicular stripes in that area (Rome et al., 1973; Pepe and Prockel, 1975; Dennis et al., 1984). C-protein can bind to portions of myosin (not the head portions), and also to F-actin (Starr and Offer, 1978; Moos et al., 1978). Therefore, C-protein may play a more significant role in thick filament function than was initially believed (Bennett et al., 1986). X-protein seems to be an isoform of C-protein, which displays many of the same characteristics (Yamamoto and Moos, 1983; Bennett et al., 1986), and is located in a band in each A-band half approximately 0.34  $\mu\text{m}$  from the middle of the A-band. H-protein is also somewhat similar to these two proteins; however, it is of a significantly lower molecular weight than that of C-protein (Starr and Offer, 1971). H-protein is located on C-protein stripe number three (0.17  $\mu\text{m}$  from the A-band center) of the thick filament, but is only seen in white fiber type muscles. The M-line is comprised of three main proteins; M-protein, creatine kinase and myomesin. M-protein and myomesin are both located at the M-line area, while creatine kinase is more of a

cytosolic protein (Turner et al., 1973; Trinick and Lowey, 1977; Grove et al., 1984).

The previously mentioned proteins comprise the myofibril. Myofibrillar tenderness is a solubilization and/or degradation of these proteins, which leads to fragmentation of the myofibrillar structure. In 1973 Parrish et al. observed the effect of postmortem aging on various properties of bovine muscle and found that there was a greater fragmentation of myofibrils near the Z-line in samples with increased sensory tenderness. Olson et al. (1976) developed the myofibril fragmentation index by using an absorbance method. These researchers related Warner-Bratzler shear data, sensory evaluation and microscopic observations of Z-line degradation to find that beef tenderness was closely related to myofibril fragmentation index values. Olson et al. (1977) continued to investigate myofibril fragmentation by observing the effects of an endogenous protease (then termed calcium activated factor (CAF)) on the fragmentation of myofibrils. They noted that CAF treatment weakened the Z-lines, and caused the degradation of troponin-T which resulted in the formation of a 30,000-dalton component. These in vitro events closely simulated the events of postmortem aging. Further evidence was added to theory of myofibril fragmentations beneficial effect on tenderness by Olson and Parrish (1977). These researchers found a direct correlation between Warner-Bratzler shear values, sensory tenderness, myofibril fragmentation index and protein degradation. Subsequently MacBride and Parrish (1977) named this kind of tenderness "myofibril

fragmentation tenderness". Recently Huff and Parrish (1993) conducted a study observing the effects of postmortem aging time, animal age and sex on the tenderness of bovine longissimus muscle. They found an increase in tenderness occurred as postmortem time increased which supports the theory of myofibril fragmentation tenderness.

### Tenderness Determination

Determination of tenderness has been an important factor in meat science for many years, and armed with the knowledge that tenderness is an important factor in consumer acceptance of meat it became important to find an objective method for the determination of tenderness of both raw and cooked forms of meat. The methods tested encompass mechanical devices, in addition to spectrophotometric detection, and sensory evaluation.

#### Shear methods

Beginning with Lehman in 1907 researchers have been using a variety of methods to estimate tenderness. Lehman (1907) used an estimation of breaking strength of meat, and the force needed to shear a meat sample past two sharp edges. A shearing device developed by Warner (1928), and Bratzler (1932) illustrated how effective their device was in estimating tenderness. This instrument was termed the Warner-Bratzler shear and has been shown to be quite highly correlated to sensory panel estimations of tenderness (range of 0.73 to 0.986; Parrish et al., 1979; MacKintosh et al., 1936), as well as highly correlated to connective tissue amounts (Ramsbottom et al., 1945).

Lower correlation values will be obtained if there is a high degree of variation within the samples tested. In one form or another, the Warner-Bratzler shear is still used today, usually the shearing apparatus is attached to a Instron Universal Testing Device. A number of other physical shearing type devices have been developed (Tressler et al., 1932; Satorius and Child, 1938; Volodkevich, 1938; Winkler, 1939; Proctor et al., 1955; Kramer et al., 1951; Miyada and Tappel, 1956a; Sperring et al., 1959; Hanson, 1972), but none have been as easy to operate, or been able to produce the same quality of reproducible results as the Warner-Bratzler shear. Smith et al. (1969) indicated a difference in shear resistance existed depending on the anatomical position within the longissimus dorsi muscle. There are conflicting reports as to the exact location of the most tender area within the longissimus dorsi muscle. Smith et al. (1969) found the medial portions to be the most tender and the lateral portions the least tender. However, McBee and Wiles (1967) reported conflicting results, showing the dorsal area to be the most tender and the medial cores to be least tender. Therefore, it is important to take a representative sample of the muscle from the lateral, central and medial areas of the steak (Smith et al, 1969). Parrish et al. (1973) tested the Armour Tenderometer which is a non-destructive measuring device for longissimus muscle still intact on the carcass; however, their results indicated that this device could only be used for very broad categories of "tender" and "tough".

### Fragmentation methods

There are a number of methods which measure a difference in the weight differential after a certain treatment has been applied to muscle. Dutson and Lawrie (1974) developed a homogenization method which was modified by Reagan et al. (1975). In this method raw meat samples were ground and blended in a high speed homogenizer. The homogenate was filtered through cheesecloth, and a portion of the filtrate was placed in a centrifuged tube and exposed to 31,000 x G for ten minutes. The supernatant was decanted and the total weight of the residue was determined and used as an estimation of tenderness. Another method used by Davis et al. (1980) utilizes frozen muscle placed in a sucrose solution and homogenized for 40 seconds. Following homogenization the solution was filtered through a series of cloth screens, and the filtrate was centrifuged at 31,000 x G for ten minutes, then the weight of the residue obtained. Davis et al. (1980) took samples all throughout the process to isolate which samples were the best estimation of tenderness. They found that the optimal sample was collected after filtration through a 250um pore size screen, and accounted for approximately 50% of the total variation in tenderness of raw beef muscle. This method was also used by Hawkins et al. (1987) who speculated that this method estimated stromal and/or myofibrillar proteins contributing to tenderness which was not related to the aging process of muscle proteins. These researchers also noted this method accounted for a significant amount of the tenderness variation.



Although this method is listed as a residual weight method, it is essentially an estimation of the myofibril fragmentation.

A method to estimate the tenderness of cooked meat using raw material and spectrophotometric equipment was developed by Olson et al. (1976). They described a method to detect changes of myofibrillar structure as observed in a myofibril solution which can be used for tenderness estimations. A basic procedure was reported by Davey and Gilbert in 1969; however, Olson and Parrish (1976) made a number of modifications to create a new procedure. In the modified procedure, four grams of meat were combined with 40 mL of an isolating medium and homogenized in a Waring blender for 30 seconds. An aliquot of the suspension was then combined with additional isolating medium (to 0.5 mg/mL final protein concentration). After a series of centrifugation and resuspension steps, this solution is measured at 540 nm using a Bausch and Lomb colorimeter/spectrophotometer. The actual myofibril fragmentation index (MFI) was obtained by multiplying the absorbance reading by a factor of 200 which created a range of approximately 30 to 100. The MFI was observed to increase during postmortem storage and was related to the Warner-Bratzler shear values collected for given muscles and stored for various amounts of time (Olson et al., 1976). Olson and Parrish (1977) conducted a study to obtain the correlations between MFI values, Warner-Bratzler and sensory tenderness. These researchers found correlation values up to 0.95 between MFI and Warner-Bratzler shears, and up to 0.97 between MFI and sensory tenderness for veal meat. Correlations were 0.73, and 0.75

for A-maturity animals, and 0.65, and 0.72 for C-maturity animals. It was determined that MFI accounts for approximately 50% of the variation in tenderness (Olson and Parrish, 1977). Culler et al. (1978) found that the MFI accounted for more than 50% of the variation in loin steak tenderness over a wide range of maturity and marbling degrees. This would indicate that MFI is an excellent source for predicting cooked steak tenderness. Parrish et al. (1979) further substantiated the earlier results which indicated the importance of MFI by finding high correlations (0.66 to 0.76) between MFI and sensory tenderness values.

#### Sensory evaluation methods

Measuring the tenderness of meat samples is a vital portion of meat science; however, no amount of objective chemical and microscopic laboratory analysis can predict the response of human subjects. For this reason many studies include sensory evaluation in addition to Warner-Bratzler shears, or chemical analysis for a complete explanation of tenderness variation. McKeith et al. (1985) evaluated the tenderness of a number of muscles in beef carcasses and used Warner-Bratzler shears as well as sensory analysis to obtain tenderness estimates. Paterson and Parrish (1986) used Warner-Bratzler and MFI, as well as a trained sensory panel for the tenderness determination of chuck muscles. Another type of panel was used by Risvik (1986). In this study a descriptive analysis team was employed and a nine point linear intensity scale was used. Overall there is a large variety of sensory panel options available to researchers and the above are just a few examples of studies using sensory panels, but

there have been countless others using a variety of sensory methods. Some of the options available are trained versus untrained panels. Training a panel group may be as simple as holding two or more training sessions where the researcher goes over the selected attributes, and exposes the panelists to extremes of each attribute, or could be more complex, possibly involving flavor profiling techniques, however this technique is more involved and is not usually involved in fresh meat sensory analysis. One study which did use a profiling technique involved the texture of restructured steaks. Berry (1986) developed a texture profile involving 10 panelists selected from 135 applicants who were screened by interest in food, concept of texture and tooth structure of the panelists. The training required four months, meeting three times per week for three hours each session. During this period the panelists determined textural characteristics, and developed the terminology and procedures for measuring the texture characteristics. This is a lengthy and involved process, but it assures researchers of consistent, repeatable results. Johnson and Civille (1986) examined the warmed-over flavor in a variety of meat products, but rather than select novice panelists, these researchers chose to use seven specialists. This dramatically decreases the training period needed to set the characteristics of the panel; however, trained experts are rather expensive as compared to non-experienced panelists. Rainey (1986) pointed out the importance of using reference standards in sensory evaluation. A reference standard should be simple, reproducible and identify only one term. A good example would be a

sucrose solution for the term sweet (Rainey, 1986). The reference standards are also useful in determining intensities and the extreme anchor points of a panel. A recent study by Medeiros et al. (1987) used three types of panels: trained, household and laboratory test market group. This type of sensory evaluation setup allowed these researchers to get a overall view of the perceptive differences between concentrate-fed cattle and range-grazed animals. It is possible to over-screen panelists and essentially isolate the panelists for a certain attribute. An example of this may be Rajalakshami et al. (1989) who isolated their panelists responses to very specific flavor profiles. So it should be apparent that there are many combinations of panels which can be used, and it is up to the researchers to determine which type of panel is best suited for their study. One main point which should be noted is a comment made by Cross et al. (1978) who stated "trained descriptive panels should not be asked to evaluate flavor or any other attribute in terms of like/dislike or acceptability". They added "these responses should be obtained only from a consumer panel". This is one area which seems to be overlooked in a number of panels.

### Tenderness Enhancement

The lack of uniform tenderness in beef is a major concern for today's meat consumers. Due to the large variety of cattle that are raised today it is unlikely that the beef industry will achieve the consistency of the poultry industry for example which only has a few breeds that are raised for meat purposes. Because consumers are

demanding a tender beef product, meat processors and researchers have tried a variety of methods to increase, or enhance tenderness. Some of the methods indirectly effect the meat, while a few of the methods have a direct effect.

### Physical/mechanical methods

One way to make a steak more tender is to inflict mechanical force via a mallet. The purpose of this form of mechanical tenderization is to achieve a breakdown of the structural constraints which serve to hold the muscle intact. These constraints include myofibrillar and connective tissue constituents. Another mechanical method, blade tenderization, is used for meat cuts targeted to become a breakfast steak type of product. Blade tenderization is also used for sectioned and formed steaks which use less tender cuts, such as the chuck, with a high amount of connective tissue (Booren et al., 1981; Rolan et al., 1988). A method of tenderization which has been used and been successful for many years is postmortem aging. Lehman was the first researcher to document the effect of storing meat prior to its consumption, and in this area was not extensively studied until the early 1970's (Ouali, 1990).

#### Postmortem aging

Postmortem aging is a process of storing carcasses and meat cuts under refrigerated conditions. Normally in beef the aging (sometimes referred to as conditioning) process takes 10-20 days, during which time proteolytic enzymes and possibly lysosomal

proteases react in the muscle to increase tenderness. What these compounds accomplish is the degradation of the normally well ordered structures, such as the actomyosin bridge formations which form permanent structures post-rigor (Honikel, 1992). Today, meat is usually aged in either vacuum bags, or on the carcass. In Europe, there is an increasing movement to use a polyvinyl box aging system rather than vacuum bags. Research shows no advantage for the European box-type aging system versus vacuum bag aging (Beekman and Parrish, 1994). Regardless of the aging system used, it has been shown that the aging, or tenderization process begins shortly after stunning (Mac Bride and Parrish, 1977; Marsh, 1983; Troy et al., 1986; Koohmaraie et al., 1987; Huff and Parrish, 1993).

#### Chilling rates

The rate that the carcass chills in the early postmortem period plays a significant role in the ultimate tenderness of meat cuts. By lowering the temperature, the rate of any biochemical reactions is slowed, which is desired for optimal tenderness. However, if the temperature is lowered to much below 6-8°C then the sarcomeres of the hanging carcasses may shorten 60 to 70%, which is termed cold shortening. Cold shortening first became apparent as a problem during the late 1950's and early 1960's in New Zealand when lamb carcasses were frozen more rapidly due to new technology in freezing methods (Locker and Hagyard, 1963). Marsh and Thompson (1958) began research in this area when it became evident that cold shortening was going to be a major problem in shipped meats. Davey and Graahuis (1976)

observed that cold shortening can occur when there is a slight drop (5°C) in temperature early in the postmortem period. The cold shortening process is most probably brought on by the lowered activity of the sarcoplasmic reticulum to bind  $\text{Ca}^{++}$  due to the decreased temperature, which causes a decrease in the ATPase activity (Davey and Gilbert, 1975). It should be noted that to some extent the cold shortening effect is reversible for a number of hours postmortem (Locker and Hagyard, 1963, Locker and Daines, 1975). Freezing functions to shorten fibers, or in other words decrease sarcomere lengths. There have been reports that decreased sarcomere lengths are linked to increased toughness (Herring et al., 1965; Marsh et al., 1968). The degree of sarcomere shortening has been shown to have an effect on the tenderness. Shortening of sarcomeres 0 to 20% resulted in no effect on tenderness, however after a 20 to 40% stretch muscles have been shown to be significantly less tender, and after a 50 to 60% stretch there is no difference in tenderness between shortened and unshortened muscles (Marsh and Leet, 1966; Davey et al., 1976). The importance of the shortening-toughening relationship is not accepted throughout the meat industry and a number of researchers doubt that a highly significant relationship exists between shortening and toughening under all conditions (Culler et al., 1978; Parrish et al., 1979; Smith et al., 1979; Seideman et al., 1987). However, almost an equal number of researchers believe that it plays a major role in the ultimate tenderness of beef (Davey and Gilbert, 1974a; Dutson et al., 1975; Honikel et al., 1986; Marsh et al., 1987). It appears that the

effect of cold shortening is dependent on the conditions the muscle is exposed to, such as the muscle being excised pre-rigor (tougher) or attached to the carcass (tender) (Goll et al., 1964). Parrish et al. (1973) have suggested that cold-shortening does not play as critical a role in the tenderness of conventionally postmortem aged beef muscle as was previously believed.

Somewhat related to the chilling rate is a practice termed hot boning (processing). This procedure is normally used in pork and involves the removal of muscles from the carcass prior to rigor onset. This process has a number of advantages such as reduced space needed for chilling, decreased amount of chilling weight loss and increased functional properties of the meat (Kastner, 1983). The major drawback of this technique is the possibility of a toughening effect due to the muscles removed pre-rigor, and no longer being attached to their skeletal restraints to prevent cold shortening (Locker, 1960). The fear of creating a pale, soft, exudative condition in hot boned pork prevents the use of electrical stimulation; however, Riette and Smulders (1989) found that no PSE conditions occurred when electrical stimulation was used on hot boned pork primal cuts.

### Electrical stimulation

In the mid 1970s the New Zealand meat industry began experimenting with electrical stimulation of carcasses to avoid the cold shortening/toughening effect (Chrystall and Devine, 1982). Electrical stimulation is now widely used due to its advantages for the beef industry which are outlined well by Savell (1979). Essentially



these improvements include increased tenderness (Cross et al, 1979; Bouton et al., 1980; McKeith et al., 1981), improvements in lean color and maturity (McKeith et al., 1981) and a decrease in the appearance of heat rings (McKeith et al., 1981; Savell et al., 1979). McKeith et al. (1981) noted there was an increase in tenderness of the deep muscles which are anatomically protected from cold shortening, and suggested that mechanisms other than the ones involved in cold protection were involved. It is agreed that electrical stimulation does affect tenderness, but what causes the induced tenderness has been the subject of a number of studies. One theory is that tenderness is increased by an intermediate fall in pH (Marsh et al., 1988; Pike et al, 1993). A second view is that a high temperature condition occurs which is thought to release proteolytic lysosomal enzymes (Dutson et al., 1982). A third theory is supra physiological contraction which causes fiber rupture and weakens the muscle tissue (Savell et al., 1978; Sorinmade et al., 1982). The true answer may be one or a combination of these theories, or a completely separate mechanism. The amount, or level of voltage, and time of application postmortem used may also play a role in the amount of tenderization in muscles. Bouton et al. (1980) determined that stimulation at low voltages (45 volts) would tenderize muscles excised after 22 hours postmortem. Extra low voltages (about 30 volts) have also been shown to be effective in tenderization, and have superior quality characteristics when compared to unstimulated sides (Powell et al., 1984; Stiffler et al., 1984). Low voltage electrical stimulation has also been used on

mature cows of 5 to 13 years of age (Hawrysh et al., 1987). The results from this study showed a slight, yet significant, increase in tenderness of several muscles due to the low voltage electrical stimulation. Powell (1991) observed the differences between animals of ages 18 months and 54 months and found that when electrical stimulation was applied in the early postmortem period, by 24 hours there was no differences in the tenderness values of the two age groups. Takahashi et al. (1987) noted a distinct difference in response to electrical stimulation depending on the frequency used. These researchers found that at two hertz, and 500 volts for five minutes there was no tissue damage and no increase in tenderness, however at 60 hertz, and 500 volts after a few seconds there was large amounts of tissue damage and a significant increase in tenderness. It was deduced that the 60 hertz frequency did not allow any relaxation between stimuli, so a supra physiological tetany situation developed. The two hertz frequency allowed for relaxation to occur between stimuli, therefore no cumulation of the tetanic response could take place. In the Takahashi et al. (1987) study it was also noted that if a rapid cooling rate in the early postmortem period occurred then the low frequency method would be effective in preventing the cold shortening response. There have also been a number of studies that have shown cases where electrical stimulation may be detrimental to tenderness (Unruh et al., 1984; Marsh, 1983; Marsh, 1985; Takahashi et al., 1987; Dransfield et al., 1992). It was stated by Takahashi et al. (1987) that if the carcass cooling rate is too slow to induce shortening then low

voltage stimulation will cause an increase in the toughness of the meat. It would appear that electrical stimulation still needs to be researched in order to determine the optimal voltage, time, and frequency needed for obtaining maximal tenderness in differing situations.

#### pH effect

The pH of meat at various times in the postmortem period has a wide range of effects on the physical and chemical properties of the final meat product. When an animal is slaughtered the blood stream is removed and oxygen along with energy carrying compounds are no longer available to the cells, also metabolites begin to accumulate in the cells. The body attempts to keep the ATP, muscle glycogen and creatine phosphate levels equal to pre-slaughter levels by using any possible reserves. Since there is no oxygen available, the muscle is forced to anaerobically breakdown the glycogen for ATP production which causes the formation of lactate. Once the glycogen stores have been exhausted there will be no more ATP production and the muscles will enter rigor mortis (Honikel, 1992). Another consequence of death is the inability to remove cell metabolites. However, the muscle cell is still able to function due to its stores of glycogen, and has ability to work in an anaerobic environment. Because the muscle cell continues to produce ATP it must also make metabolic end products, which in anaerobic situations is lactic acid. The lactic acid formation and accumulation in the muscle causes the pH to fall from about 7.0 in the immediate postmortem period to an ultimate postmortem pH of 5.3 to 5.8 after 18

to 40 hours (Honikel et al., 1986; Honikel, 1992). Some researchers believe that three hour pH is a good indicator of the ultimate muscle tenderness (Smulders et al., 1990; Pike et al, 1993). Their theory is that the muscle glycolytic rate plays a key role in the ultimate tenderness by allowing meat to reach a three hour pH of approximately 6.1 which is required for maximal tenderization. It has been shown that high postmortem temperatures will accelerate the glycolysis cycle, while lower temperatures will slow the process (Busch et al., 1967; Bendall, 1978). In certain carcass situations, it is beneficial to avoid a rapid chill of pre-rigor muscle in light weight carcasses with little insulation, otherwise the muscle will contract due to high ATP levels and cold shortening can occur. Using various levels of electrical stimulation, Marsh et al. (1987) demonstrated that for optimal tenderness, an intermediate rate of glycolysis with a three hour pH of 6.1 was most desirable. It was also found that if the three hour pH was higher or lower than 6.1, a decrease in tenderness would result. Research by Smulders et al. (1990) substantiated the Marsh results, and also discovered that sensory tenderness was influenced by shortening only in muscle which had a slow rate of glycolysis (three hour pH of 6.3 or higher). Busch et al. (1967) found no relationship existing between the ATP level (glycolytic rate), pH and tenderness in bovine muscle. Other researchers who do not believe that three hour pH is a key factor in determining tenderness are Dransfield and Etherington, (1988) and, Purchas (1990). Purchas (1990) believes that the ultimate pH, not the three hour pH, is related to tenderness. Purchas found that an ultimate

pH of 6.1 was toughest and shear forces decreased as the pH traveled in either direction. Jeremiah et al. (1991) substantiated the Purchas results, and found toughest samples in the pH 5.8 to 6.2 range. It would appear that both theories have merit and at this point neither one can be discounted. More research is currently underway, but it seems that at least a portion of the variation in meat tenderness can be controlled by the glycolytic rate of the muscles.

#### Enzymatic tenderization

The enzymes involved in tenderization can be separated into plant proteins and natural occurring enzymes which are in the muscle tissue. Proteolytic enzymes from plant proteins such as papain have been used for a number of years to tenderize meat products which are known to be tough. It has been reported that papain hydrolyzes actomyosin to peptides and digests connective tissue both of which result in increased tenderness (Miyada and Tappel, 1956b; Wang et al., 1957; Kang and Rice, 1970). The derivatives of these proteins can be injected into beef animals prior to slaughter or applied to the surface of the meat shortly before it is to be prepared for consumption. The papain activity is limited at refrigerated temperatures (5°C), and reaches its optimal activity at 65°C (Ockerman, 1991). Papain appears to effect actomyosin by degrading myosin more readily than actin, and will slowly hydrolyze collagen (Tsen and Tappel, 1959). Other proteolytic enzymes of plant protein origin such as bromelin and ficin have also been used. According to El-Gharbawi and Whitaker (1963) elastin does not need to be denatured prior to ficin and bromelin addition; however,

collagen must be denatured before these enzymes will be effective as tenderization enhancers. However, the use of these enzyme products has been limited to meat which is known to be tough, and is not extensively used in the meat industry. Of a greater interest to meat scientists are the activity of two enzyme systems: lysosomal enzymes of the cathepsin group, and calcium dependent proteinases (calpains). The lysosomal cathepsins are localized inside the membrane compartments (lysosomes) of muscle, and are probably released during low pH and high temperature conditions from these compartments to act on myofibrillar proteins (Dutson, 1983). The most characterized of the cathepsins are B, D, H, and L (Koochmaraie, 1988), although a total of eight lysosomal proteases have been found in skeletal muscle (Goll et al., 1989). A number of studies have observed the action of various cathepsins on isolated myofibrils, and found several myofibrillar proteins were degraded, and all the cathepsins tested degraded actin and the myosin heavy chain (Ouali et al., 1982; Goll et al., 1983; Zeece et al., 1986). Calkins and Seideman (1988) found the total activity of cathepsins B and H was correlated to the increase in tenderness from three to six days postmortem when Warner-Bratzler shear values were compared with the activities of cathepsin B and H. However, Etherington et al. (1987) could not find any relationship between the rate of tenderization and cathepsin B and L activity. Olson and Parrish (1977) did show that under normal refrigerated postmortem storage conditions actin and myosin were not degraded. Uytterhaegen et al. (1994) conducted a study which illustrated no changes in texture or

proteolysis when cathepsin inhibitors were added to meat samples, which suggests the cathepsins play a minor role in the tenderization process. It is difficult to conclude from a reviewing standpoint that the cathepsins are directly related to tenderness, due to the number of conflicting studies. It does appear that under "normal" conditions the cathepsins do not have a significant tenderization effect.

The other main enzyme system believed to be involved in the tenderization process are the calpains which have also been called: kinase activating factor (KAF), calcium dependent proteases (CDP), calcium activated factor (CAF), calcium dependent neutral proteases (CDNP), calcium activated sarcoplasmic factor (CASF) and calcium activated proteases (CAP). Busch et al. (1972) found that rabbit skeletal muscle contains an endogenous protein capable of Z-line removal which was termed CASF. This sarcoplasmic factor was hypothesized to be activated when calcium was released from the sarcoplasmic reticulum. It has been suggested that these proteinases also promote protein turnover of thick and thin filaments (Dayton et al., 1975; Goll et al., 1989). Initially, calcium activated factor (CAF) was found to degrade the Z-lines of isolated myofibrils which increased tenderness when chemical conditions were used to mimic postmortem events (Olson et al., 1976). Olson et al. (1977) proved the disappearance of troponin-T corresponded to a subsequent appearance of the 30,000 dalton component by treating purified troponin with purified CAF. These researchers also noted that CAF weakened the Z-lines by removing  $\alpha$ -actinin. MacBride and Parrish (1977) conducted a

study to observe any chemical differences between tough and tender longissimus muscles. These researchers discovered that the 30,000-dalton component was related to tender steaks, and highly fragmented myofibrils. They also suggested the use of the 30,000-dalton component as a detector of tenderness by one day postmortem, and suggested "myofibril fragmentation tenderness" as a way to explain tenderness.

The calpain enzyme system is comprised of three main units: u-calpain, m-calpain and the calpain inhibitor calpastatin, as well as n-calpain and possibly one more regulatory protein (Goll et al., 1992). The difference between the three proteases is their  $\text{Ca}^{2+}$  requirements. The u-calpain component requires 3-50  $\mu\text{M}$   $\text{Ca}^{2+}$ , and m-calpain needs 200-1000  $\mu\text{M}$   $\text{Ca}^{2+}$ , while n-calpain needs about 3000  $\mu\text{M}$   $\text{Ca}^{2+}$  to achieve half maximal activity (Goll et al., 1991; Goll et al., 1992). Calpastatin also plays a key role since at  $\text{Ca}^{2+}$  levels which activate calpains, calpastatin may inhibit, or at least slow down the calpains activity (Nakamura et al., 1989; Koohmaraie, 1992). There have been two recent models proposed for the activation of calpains, which tend to agree with one another. The model of Dransfield (1993) may be a bit simplistic but it is easily understood. Dransfield (1993) states that the tenderization of meat is related to the calpains activity which is dependent on the temperature, pH and the level of free activated calpains. This level is determined by the balance which exists between the decay, the release from calpastatin and the inactivity (in presence of calcium) of the free activated calpains. Goll et al. (1992) proposed a



model which is quite detailed and complex. Goll et al., (1992) suggested the regulation involves specific responses to calcium ions at four calcium binding sites on the calpain molecules. Geesink (1993a) noted that a high ultimate pH accelerated the breakdown of titin and nebulin due to enhanced activity of u-calpain. When the calpains are autolyzed (which is stimulated by the presence of calcium) the mass of the protein decreases, and the amount of calcium needed to achieve half maximal proteolytic activity is lowered (Inomata et al., 1985). The autolysis of calpains results in a change in its conformation in that a slight lengthening of the molecule occurs; however, the  $\alpha$ -helical and  $\beta$ -sheet structures which contain the active sites, are kept intact (Edmunds et al., 1991). Kapprell and Goll (1989) demonstrated the complete inhibition of either autolyzed or unautolyzed forms of u-and m-calpain at calcium ion concentrations which are lower than needed for autolysis or proteolytic activity. A rather large percentage of the tenderness variation (approximately 90%) can be attributed to calpain tenderization of meat according to Goll et al. (1991). This is a huge percentage considering all of the factors involved in the tenderization process, although a number of studies have observed the degradation of myofibrillar components due to the calpains and have found they effect a large portion of the myofibril (Dayton et al, 1976; Olson et al., 1977; Lusby et al., 1983; Ouali et al., 1983; Robson et al., 1984). Use of immunofluorescence and immunogold localization has led to the conclusion that the calpains and calpastatin are located intracellularly in the myofibrils, nuclei and mitochondria, and very little if any of the

enzymes are located in the cytoplasmic space between the myofibrils (Kumamoto et al., 1992). Recently Uytterhaegen et al. (1994) compared calpains and cathepsins by the use of specific inhibitors and found that the calpains were the proteases involved in beef tenderization. This area of research has contributed substantially to understanding tenderization. Because of the intellectual and practical challenge of tenderness research, it will continue to be an active area of research.

#### Chemical treatments

It has been well documented that meat tenderness will increase during postmortem storage and will be completed in eight to 14 days (Dransfield et al., 1980); however, Huff and Parrish (1993) found a tenderization effect extending to 28 days postmortem. Certain meat cuts will always be tough to a certain degree due to their physical composition, for example certain muscles in the beef chuck, e.g. supraspinatus, will be tougher than the tenderloin. Therefore, the meat industry continues research to increase the value of certain muscles by accelerating the aging process. One way to increase the value of meat to all segments of the industry is the addition of certain chemical compounds to the meat in an attempt to enhance specific palatability traits. The main meat quality attributes affected by the addition of various salt type compounds are water holding capacity (WHC) and tenderness.

### Sodium chloride (NaCl) and phosphates

The effects of NaCl and condensed alkaline phosphates on meat, and the mechanism involved have been very well documented over the years (Swift and Ellis, 1956; et al., 1957; Hamm, 1960; Shults et al., 1972; Offer and Trinick, 1983; Trout and Schmidt, 1983; Paterson et al., 1988). These researchers have shown that the addition of NaCl causes an increase in the ionic strength and pH of the meat which in turn affects the WHC of the muscle. The end result of this situation is an increase in the electrostatic repulsions of muscle proteins, therefore a relaxing of the myofibrillar structure results and more water can be trapped (Hamm, 1960). It appears that there are several mechanisms by which phosphates can increase the WHC of meat. Hamm (1960) demonstrated the effect of pH by observing an increase in the WHC when the alkalinity was increased by adding alkaline phosphates to meat. Hamm and Grau (1955) discovered that sodium would displace calcium which was bound to the meat, and Trout and Schmidt (1983) reported a dissociation of the actomyosin complex, a chelation of divalent metal ions, and binding of proteins with phosphate anions leading to an increase in WHC. Hellendoorn (1962) reported that tripolyphosphate had the highest WHC enhancing ability of any phosphates tested, and was very effective in water retention in meats in conjunction with NaCl. It was also noted by Shults et al. (1972) that tripolyphosphates were the most effective phosphate in reducing meat shrinkage when heated at 70°C. A study reported by Offer and Trinick (1983) using phase contrast microscopy and SDS-PAGE found a swelling

effect on myofibrils when NaCl was added; however, when pyrophosphates were added in conjunction with NaCl there was swelling, but less NaCl was required to gain the same degree of swelling as was observed with NaCl alone. It was also noted that a higher amount of protein extraction from the A-band area occurred when NaCl and pyrophosphates were used versus NaCl alone. These observations led to the conclusion that the effects of NaCl and pyrophosphate were due to Z-line, M-line and actomyosin crossbridge solubilization resulting in greater myofibrillar space, and increased WHC (Offer and Trinick, 1983). Paterson et al. (1988) demonstrated the effects of NaCl and pyrophosphates on the muscle proteins titin and nebulin on WHC. The results of their study supported those of Offer and Trinick (1983), plus they noted an extraction of titin when NaCl and polyphosphate were present, and concluded that the removal of titin allows more space for the myofibril to swell, because titin is thought to comprise a third filament structure. Knight and Parsons (1988) observed the actions of myofibrils at various salt and polyphosphate conditions and found the optimal salt level for swelling to be approximately one molar (5.8%). If a five molar (29%) solution was used very little if any swelling was detected, and these myofibrils lost their ability to react to the addition of polyphosphate.

#### Calcium chloride ( $\text{CaCl}_2$ )

The relationship of calcium with tenderness has been researched for a number of years, and most of the studies have found a tenderizing effect results when a calcium solution is added to the meat system.

Busch et al. (1972) found that calcium concentrations of greater than 0.1 mM resulted in Z-line degradation. Cheng and Parrish (1977) found evidence to support the theory that calcium acts to tenderize meat, when they added calcium and chelated calcium present in the muscle with oxalate and observed increased protein degradation in treated muscle by using SDS-PAGE. Palladino and Ball (1979) found some tenderizing effect due to  $\text{CaCl}_2$  in poultry muscles from spent hens, however NaCl appears to have the most beneficial effect on poultry meat. Koohmaraie et al. (1988a) undertook incubation studies with bovine longissimus muscle and solutions of  $\text{CaCl}_2$  and EGTA/EDTA. These researchers concluded that postmortem tenderization events are calcium mediated, and calcium dependent proteases were responsible for the tenderization which occurred. Koohmaraie et al. (1988b) were the first study to infuse  $\text{CaCl}_2$  into a carcass in an attempt to increase tenderness. Lamb carcasses were infused with a 0.3 molar  $\text{CaCl}_2$  solution in conjunction with electrical stimulation immediately after death. From their results, it was concluded that the optimal treatment was low frequency electrical stimulation followed by infusion with  $\text{CaCl}_2$  to achieve maximum tenderness at 24 hours postmortem. Koohmaraie et al. (1989) infused various levels of  $\text{CaCl}_2$  solutions and found a solution of 0.075 molar had no effect on tenderness, but if a 0.15 molar solution was infused a significant decrease in the shear force values resulted and that the 0.3 molar had the lowest shear force values. Koohmaraie et al. (1989) concluded that the  $\text{CaCl}_2$  tenderization is due to the effects of calcium dependent proteases

(calpains) rather than ionic strength (higher molarity solutions causing degradation/solubilization of proteins). This research was extended to ground lamb meat by St. Angelo et al. (1991). These researchers saw indications that infusion with a 0.3 molar  $\text{CaCl}_2$  solution increased lipid oxidation and warmed over flavor development; however, addition of maltol and/or sodium ascorbate (antioxidants) to the  $\text{CaCl}_2$  solution counteracted the detrimental effects, while not interfering with the positive tenderization effect of  $\text{CaCl}_2$ . Morgan et al. (1991a) injected 0.3 molar solutions of  $\text{CaCl}_2$  within 30 minutes of slaughter and noted a decrease in shear values, shorter sarcomere lengths and lower amounts of connective tissue. It was speculated that sarcomere shortening may have altered the matrix structure of the connective tissue. Other theories of calcium's role in the myofibril include Taylor and Etherington (1991) who incubated myofibrils with calcium and saw no solubilization of Z-line proteins ( $\alpha$ -actinin, desmin), but rather a solubilization of C-protein, troponin I, troponin T and tropomyosin. They also noted that inclusion of proteinase inhibitors (E64, leupeptin, pepstatin) did not prevent protein solubilization, and concluded that solubilization of proteins by calcium ions was due to a salting out reaction rather than proteolytic actions. Wheeler et al. (1991) used round muscles (biceps femoris and semimembranosus) from *Bos indicus* cattle and late-castrated steers to observe the effects of  $\text{CaCl}_2$  (0.3 molar) injection. Results indicated that hot boning had no effect on the shear values; however, hot boning in conjunction with  $\text{CaCl}_2$  injection resulted in increased tenderness. These researchers observed an

increase in tenderness by one day postmortem, and concluded that the calpain enzyme system was the agent of tenderization. Tatsumi and Takahashi (1992) examined isolated chicken breast myofibrils treated with a 0.1 molar solution of  $\text{CaCl}_2$  including leupeptin to prevent protease activity. Nebulin was the main protein of interest, and they detected a non-enzymatic degradation of nebulin into smaller subunits. It was concluded that since nebulin is linked to thin filaments that this calcium induced degradation plays a role in postmortem tenderization. Marinations with  $\text{CaCl}_2$  have also been used in an attempt to enhance tenderness by Whipple and Koohmaraie (1993); they collected steaks from various types of cattle at five days postmortem, and marinated them in a 0.15 molar  $\text{CaCl}_2$  solution. The results indicated that marination was effective as a tenderness enhancing agent, and may be useful just prior to preparation for the consumer. Wheeler et al., (1993) compared pre-rigor and post-rigor treatments of  $\text{CaCl}_2$  at various concentrations (0.175, 0.2 and 0.25 molar) and observed that not only did an increase in tenderness occur, but that there was no major off flavor or color development. From the results, it was concluded that an injection of  $\text{CaCl}_2$  at 24 hours postmortem at a level of 0.2 molar and 5% (wt/wt) provided optimal results. Geesink (1993b) conducted an experiment to investigate the cause of the  $\text{CaCl}_2$  tenderization effect. The two possible causative effects observed were calpain activity and extreme contraction. He found that there may be an ionic strength and enzyme cooperativity in tenderizing activity. Tenderness is a very complex process which appears to involve a

number of factors. The studies mentioned earlier in this review indicate the importance of tenderness research and it is almost a certainty that more studies will be undertaken in an attempt to isolate the effective agent in the tenderization process. When all the studies involving tenderness are evaluated the majority, either directly or indirectly, involve a fragmentation of the myofibrils. It would appear that the fragmentation of myofibrils caused by the inherent protease calpain is the main tenderization effect, and studies conducted in the future will in one form or another involve the analysis of muscle fragmentation, calpains and their inhibitor, calpastatin.

#### Microscopic Evaluation of Tenderness

The three main attributes of meat which directly relate to the eating quality are tenderness, juiciness and flavor. Of these attributes, tenderness has received the major portion of researchers' attention. Researchers have examined nearly every instrument to observe tenderness and tenderization. A popular way of studying tenderization has been the use of microscopy. There are many ways microscopes can be utilized in tenderness studies, such as simple viewing of aging effects under phase contrast conditions, or as complex as gold labeling samples for electron microscopes. The main microscope systems used are electron microscopy, X-ray diffraction, phase contrast and immunofluorescence microscopy, and their modifications.



### Myofibrillar structure

The microscope has been a very useful instrument in studying the structured elements of the muscle cell. The functional part of skeletal muscle is the muscle cell which is also termed a muscle fiber. These are long slender cells which taper at each end, and contain all the subcellular components. Skeletal muscle fibers rarely, if ever, branch and range in length 1-40nm with an average of about 25nm (Goll et al., 1984). Muscle fibers are composed of subcellular structures called myofibrils. The myofibril contains the contractile elements of the muscle, and each fibril appears to extend throughout the entire length of the fiber (Huxley, 1972). The myofibrils are lined up parallel to the muscle fibers and are not surrounded by a membrane, and are comprised of hundreds to thousands of repeating units joined by Z-lines, which are termed sarcomeres. The following information is from two excellent reviews of the muscle system (Huxley, 1972; Goll et al., 1984). The structure of the myofibril is amazingly regular in its banded appearance which is in perfect register. The banding pattern is due to the organization of protein filaments which comprise an overlapping structure. The banding or striated appearance is due to the birefringent capabilities of the bands. The dark bands viewed under a light phase contrast, or electron microscope are termed the A-band, which stands for anisotropic (birefringent), and the lighter bands are named I-bands, which refers to the isotropic (weakly birefringent) nature of this band. By using phase microscopy these two bands are instantly visible and create the uniform light and dark banding appearance which is

associated with striated muscle. Lighter I-bands are perpendicularly bisected by a dark line which represents the Z-line, or Z-disc. The average distance from one Z-line to the next Z-line (sarcomere length) on the myofibril is approximately 2.5 $\mu$ m in a resting mammalian skeletal muscle. When the muscle contracts, the sarcomere length can decrease to below 2.0 $\mu$ m, to a maximal contractile length of 1.8 $\mu$ m in living cells. Ultrastructurally, thick filaments (composed primarily of myosin) extend the length of the A-band; however, the thin filaments (composed mainly of actin) do not extend far enough to meet at the center of the A-band, thus creating the H-zone which is the perpendicular area between the thin filament ends. The H-zone appears a bit lighter within the darker A-band, and has a very dark line bisecting it which is termed the M-line. Thin filaments extend from the edge of the H-zone to an attachment within the Z-line. Thick filaments may be aligned and anchored by proteins within the M-line, and also have protrusions extending outward at a perfectly regular interval (approximately every 400 Angstroms) termed cross bridges. When an electron microscope is used, it is also possible to see a structure termed the pseudo H-zone which is the perpendicular area on the thick filaments containing no cross-bridges. The cross bridges are, in part responsible for the contractile process because they attach and detach to the thin filament creating the rowing movement associated with muscle contraction. Cross sectioned myofibrils have a very regular structural apparatus. Cross sections through the H-zone illustrate a hexagonal array of the thick filaments held in line by a

series of protein cross bridges termed M bridges. When a cross section through the overlap area of the thick and thin filaments is observed each thick filament is surrounded by six thin filaments in a hexagonal array around the thick filament. Furthermore, each thin filament is surrounded by three thick filaments, so in any given plane of vertebrate skeletal muscle, there is a 2:1 ratio of thin to thick filaments. In the I-band region, the thin filaments lose the hexagonal array and assume a square lattice formation as they near the Z-line. The structure of the sarcomere and, in turn, the myofibril allow for the regular contraction of the muscle fiber to occur via the sliding of the thick and thin filaments (Huxley, 1986; Huxley et al, 1987). Recently, the sliding filament theory has been questioned and in some researchers views the exact contractile mechanism remains unknown (Pollack, 1988; Morel and Merah, 1992; Schutt and Lindberg, 1992). Pollack (1988) raises the following questions about the presently accepted contractile process: 1) absence of proof of cross-bridge rotation, 2) inability to explain stepwise shortening on a whole muscle scale, 3) total absence of cross-bridge tension noise, 4) possibility of tension beyond overlap, and 5) observations of thick filament shortening. It appears that the accepted cross-bridge theory of contraction may need to be modified in the future.

### Sarcomere length

It was mentioned earlier that the sarcomere length will change depending on the contractile state of the muscle. A number of postmortem studies have attempted to relate the sarcomere length of a

given muscle to the ultimate cooked tenderness of that muscle. The sarcomere lengths in nearly all of the following studies were determined with electron microscopy; however, light, and confocal microscopes were also used in conjunction with antibodies to certain proteins. Locker (1959, 1960) conducted a series of studies examining the contractile state of various muscles and how each state related to tenderness. Locker concluded that relaxed muscles were more tender than muscles in a contracted state, and, depending on the connective tissue amounts, may play a large role in overall tenderness determination. Locker and Hagyard (1963) conducted experiments to observe the effect of cold shortening because it seemed sarcomere length played a role in tenderness. They were unable to discover the cause of cold shortening of muscles, but again emphasized the importance of contractile state to ultimate tenderness. Herring et al. (1965) conducted a study to observe the differences in sarcomere length and tenderness when carcasses were stored in various positions. These researchers concluded that positioning the carcass in a vertical fashion versus a horizontal position caused certain muscles to stretch (psoas major, latissimus dorsi and rectus femoris) while other muscles would shorten (longissimus dorsi, gluteus medius, adductor, biceps femoris and semitendinosus), thus creating shorter sarcomere lengths. Herring et al. (1965) also found a correlation (-0.46) between sarcomere length and tenderness, and concluded that sarcomere length in conjunction with fiber diameter are key constituents contributing to tenderness. Hostetler et al. (1970) hung carcass sides from the

obturator foramen versus the achilles tendon and found improved tenderness, and longer sarcomere lengths in longissimus dorsi, semimembranosus and semitendinosus muscles. In another study examining suspension differences, Hostetler et al. (1972) investigated several carcass suspension methods which could cause a stretching of the sarcomeres of major muscle groups. Of the five suspension methods tested, the hip-free (obturator foramen hung) carcasses had greatly improved the tenderness in major muscles of the round area. Marsh and Leet (1966) illustrated, by fixing a pre-rigor excised muscle, that even if both ends of the muscle are fixed, shortening-induced toughness still occurs. They found a compensating reaction existed within the muscle, so if an area of the muscle shortened, a separate area of the muscle would lengthen. The meat industry never utilized the carcass hanging information, so for all practical purposes, it is not researched at this time. A number of other studies have also linked tenderness to sarcomere lengths in a variety of muscles. McGinnis et al. (1989) found poultry anesthetized by sodium pentobarbital versus normal slaughtering procedures, had longer sarcomere lengths, and were significantly more tender. Bilgili et al. (1989) experimented with chicken pectoralis major muscle and indicated that postmortem aging temperatures affected the sarcomere lengths. Lower temperatures in the 0 to 14°C range, resulted in the shortest sarcomeres and the highest shear values. Smulders et al. (1990) created and observed a wide range of sarcomere lengths in meat by the application of electrical stimulation, and various chilling cycles. They found that in

carcasses with rapid glycolysis, there was a very strong relationship between sarcomere length and tenderness, however in the slow glycolytic samples, very little relationship was observed. Beekman and Ringkob (1992) found a significant correlation between sarcomere length and tenderness in both steers and bulls and also noted that a difference exists in the sarcomere lengths of single versus multiple myofibrils. They found that there was a significant difference ( $P < .01$ ) in the measurements of a single myofibril (shorter), versus a myofibril that was comprised of two or more myofibrils (longer). It should therefore be noted which type of myofibril is being measured.

#### Microscopic evaluation of gap filaments

There are some filaments, besides the thick and thin filaments, which have been found to exist within the myofibril and extend from the Z-line to the M-line area. These filaments may play a role in contributing to meat toughness. Huxley and Hanson (1954) used microscopy to discover a type of filament structure, termed S filaments. Sjostrand (1962) was the one of the first researchers to identify these new filaments by hyper-stretching frog semitendinosus muscles, creating a gap between the ends of the thick and thin filaments. Very thin filaments (thinner than the I-band filaments) were seen to exist in the created gap area and had a diameter of roughly 30 angstroms. Locker and Leet (1975) stretched beef sternomandibularis fiber strips to approximately five times the resting length which produced a two  $\mu\text{m}$  gap between the A and I-bands. These researchers found gap filaments existing in this area which seemed to

be continuous with the thick filaments, thus allowing tension to be maintained. In an extension of their first study, Locker and Leet (1976a) further investigated the gap filaments, and discovered when actin and myosin were extracted from highly stretched fibers, these gap filaments extended from an overlap at the center of the sarcomere, through the Z-line, suggesting these filaments formed the core of the thick filaments. These researchers also noted when the fibers with no actin or myosin were released from their stretch position, they always sprang back to their original length, thus indicating the gap filaments were involved in a restoration of force reaction due to an apparent elastic nature. Further studies by Locker and Leet (1976b) on gap filaments with highly stretched post and pre-rigor muscles showed that gap filaments apparently caused the M-line to be pulled off center in the same manner in connecting sarcomeres, suggesting a linkage between the A-bands. In 1977, Locker et al. summarized their findings by stating that a third set of filaments termed "gap filaments" did exist, and were responsible for setting the limits for tensile strength of the myofibril from the raw state to highly cooked conditions. In this early period of gap filament research, not all scientists were convinced that these filaments actually existed. Ulrick et al. (1977) conducted studies and concluded that gap filaments existed only in invertebrate flight muscles and not in vertebrate muscle, which was contrary to the early work of Locker et al. (1976; 1977). Remedios and Gilmour (1978) studied gap filaments by first extracting the thick and thin filaments and then using electron microscopy to observe gap filaments. These

researchers also conducted solubility studies and concluded that the gap filaments were composed of connectin (titin). Locker and Daines (1980) hypothesized that gap filaments are indeed composed of connectin (titin), and postmortem tenderization occurs due to the actions of cathepsins on the gap filaments. Locker (1982) proposed a model for the gap filament orientation in the myofibril which instantly was attacked for being too simplistic. In his model, Locker had the gap filaments acting as a core to two thick filaments of adjacent sarcomeres, linking the thick filaments through the Z-line. This theory explained the observed pulling of alternate thick filaments toward opposite Z-lines. Orcutt and Dutson (1985) did not observe the pulling effect, but did see a higher degree of gap filaments at pH 7.4 versus pH 5.5 and concluded that gap filaments may be rapidly degraded in postmortem muscle. Locker (1987) silenced the majority of his critics by updating his model of gap filament location within the myofibril. In this model, the gap filaments, now renamed the T-filaments (for titin) were comprised of single titin molecules which span half of the sarcomere from Z-line to M-line. Locker further suggested that there are six T-filaments lying longitudinally along the surface of the thick filament, and covered by C-protein molecules in axial and transverse rows. The C-protein serves to bind the T-filaments to the thick filament, and therefore interferes with the antibody response of titin in this overlap area. Locker also stated in this study that the T-filaments come together in the I-band region to form the gap filaments. This is essentially the model used today, although now the filaments



are less often referred to as gap, or T-filaments , but rather, titin filaments, or simply titin.

#### Microscopic observation of titin

There have been several relatively new myofibrillar proteins which have been studied using a variety of microscopic techniques. One of these proteins which was mentioned previously, and comprise the gap filaments, is titin. When titin was discovered by Wang and co-workers, (1979) a number of laboratories began in depth titin research. Initial electron microscopy of muscle showed titin molecules to be long, elastic type structures about three nm in diameter with a length which can vary from 100 to 1500 nm (Maruyama et al., 1984; Trinick et al., 1984; Wang et al., 1984a). Wang et al. (1984a) developed four monoclonal antibodies to titin which stained four paired bands from the M-line area to just beyond the A-I junction, which suggested a single molecule extending from the M-line to the I-band. Hill and Weber (1986) developed three monoclonal antibodies for chicken titin, and used these in conjunction with indirect immunofluorescence and observed specific binding of the three antibodies in the A-I junction. The researchers also used immunoelectron microscopy to detect titin on the end of the myosin filaments. Several researchers have used rotary shadowing and electron microscopy to observe the beaded structure of titin which may exceed a micron in length (Trinick et al., 1984; Wang et al., 1984b; Maruyama, 1986). Paterson et al. (1988) used phase contrast microscopy in conjunction with SDS-PAGE to illustrate the increased extraction of titin when NaCl and phosphates were added

to beef muscle. Ringkob et al. (1988) used a monoclonal antibody developed by Wang and Greaser (1985) and noticed the bands went from two per sarcomere to four per sarcomere during postmortem aging. Results also suggested a conformational change occurred in the early postmortem period. Hainfeld et al. (1988) used scanning transmission electron microscopy to investigate the mass of titin and created a mass to length ratio for titin which lead these researchers to suggest titin had a mass of approximately 2.4 million daltons and a length of over one micron. Horowitz et al. (1989) used immunoelectron microscopy to illustrate that titin remained bound to thick filaments during contraction, and the elastic properties of titin were not altered by cross bridge activity. Nave et al. (1989) used metal shadowing and electron microscopy to obtain micrographs of the titin molecule. They saw a single globular head at the end of a long thin rod, and usually two to three titin molecules were linked together at the head region. These researchers were also able to show that the head regions are located at the M-line area, and the free end of the molecule extended to the Z-line area. Furst et al. (1989) were able to provide evidence for titin and thick filament binding by using immunoelectron microscopy. They detected a 42 nm repeating sequence which matched banding patterns of the A-band. Beekman and Ringkob (1992) dual labeled myofibrils with antibodies to titin and  $\alpha$ -actinin (the Z-line reference) and used the microscopy technique of indirect immunofluorescent to show the relationship of certain titin epitopes to the Z-line. Microscopic observation using dual labels allows for a direct Z-line reference

which can be superimposed onto the fluorescence from the titin antibody, thus creating a single image rather than a phase contrast image and a fluorescent image. It is without doubt that the microscopic studies will continue to shed light on the locations of not only titin, but other myofibrillar/cytoskeletal proteins as well, such as nebulin, desmin and synemin.

### SDS-PAGE Analysis of Tenderness

A most efficient way of tracking/monitoring proteins in meat systems is to collect a sample and prepare it for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Sodium dodecyl sulfate (SDS) serves to "buffer" the charges of proteins by negating the charge to mass ratio, therefore all the charges are essentially equal (Huff-Lonergan et al., 1994b). It will also denature and form a long bonded complex with each protein molecule. The SDS is incorporated into the gel buffers, electrode buffers and added to the proteins via a heated solution. The polyacrylamide gel has pre-determined gel pore sizes (created by the ratios of acrylamide and bis-acrylamide) which allow the proteins to migrate solely due to the size of the proteins polypeptide chain, and the derived molecular weights are quite accurate (Huff-Lonergan et al., 1994b). SDS-PAGE is ideally suited for the determination of myofibrillar/cytoskeletal proteins, or their degradative products, remaining after a given treatment. Nearly all meat research uses the SDS-PAGE procedure of Laemmli (1970), or a similar method with slight modifications.

### Protein degradation/purification/isolation

Protein degradation is probably the most important activity which must occur for increased tenderness, and one way to detect any degradation of a given protein is by using SDS-PAGE. Olson and co-workers (1977) developed a SDS-PAGE system in their study to observe the effects of calcium activated factor on beef muscle myofibrils and MacBride and Parrish (1977) used the SDS-PAGE techniques of Olson et al. (1977) in their study to observe protein changes in myofibrils from tough and tender muscles. These researchers found an interesting degradative product by using SDS-PAGE which appeared as the troponin T band disappeared in tender muscles but not tough muscles. They termed the product the 30,000 dalton component and proved that it was indeed a degradative product of troponin T by catalysis with CAF. Wang et al., (1979) provided proof of titin's existence using SDS-PAGE as well as immunofluorescence staining. The SDS-PAGE results from this study showed two very large bands in the gels above the myosin heavy chain band. Maruyama et al. (1981) used SDS-PAGE to track the effects of various proteolytic enzymes (trypsin, chymotrypsin, papain, pepsin, calcium activated neutral protease and nagarse) on titin. These researchers also found that titin and connectin were most probably the same protein. Lusby et al. (1983) aged bovine longissimus muscle for various postmortem periods and observed the changes which occurred using the Studier (1973) SDS-PAGE method. They found that titin degradation was both time and temperature dependent based on a gradual disappearance of titin bands, i.e., at higher temperatures and

longer times, there was less dense bands of titin on electrophoresis gels. Davey (1983) wrote an informative review article on the process of meat aging and the effects aging has on connectin, desmin, proteins of the Z-line, thin filaments and thick filaments. It was pointed out that aging studies are quite complex due to factors other than protein degradation which contribute to tenderness. These other factors, such as contractile state and collagen, can mask the increase in tenderness caused by proteolytic action; however, MacBride and Parrish (1977) found no significant differences in intramuscular collagen amounts, sarcomere length and intramuscular fat between tender and tough meat samples. Bechtel and Parrish (1983) designed a study to observe the effects of postmortem storage time and temperature on muscle. SDS-PAGE analysis allowed the researchers to track protein degradation and find that at a temperature of 37°C myosin heavy chain would be degraded after one day postmortem, but at 4°C there was little degradation after storage times as high as 14 days. Koohmaraie et al. (1984) conducted a similar study and noticed the appearance of a 95,000 dalton component, and the 30,000 dalton component. They also noted an increased appearance of a band at 55,000 daltons, and the gradual appearance of a 110,000 dalton component. Paxhia and Parrish (1988) tested poultry and pork muscles for postmortem storage effects using SDS-PAGE. They found titin and nebulin were degraded in a more rapid fashion in poultry light muscles versus dark muscles; however, titin and nebulin in pork dark muscles were degraded more rapidly. Another study observing protein degradation was reported by Anderson

and Parrish (1989). In this study, the degradation of titin and nebulin from tough and tender longissimus muscle were tracked with SDS-PAGE. It was observed that titin and nebulin bands were less intense in tender muscles. Ouali (1990) illustrated that proteolytic fragments can be observed with SDS-PAGE a few hours post-slaughter thus supporting the idea that aging begins in the early postmortem period. Koochmaraie (1992) has used SDS-PAGE techniques in an attempt to identify the effects of autolyzed calpains on muscle proteins by noting the size of the autolyzed fragments and their corresponding activities. SDS-PAGE is also useful in detection of proteins which are being purified, or classified. Wang et al. (1984b) used SDS-PAGE and electron microscopy in order to characterize titin as an extremely long elastic protein which exists in a doublet form. Trinick et al. (1984) conducted studies with SDS-PAGE to purify and determine some properties of titin. Paterson and Parrish (1987) investigated SDS-PAGE conditions needed to identify tender versus tough muscles as dictated by titin and nebulin amounts. A ratio of acrylamide to bis-acrylamide of 37:1 with a gel buffer at pH 8.0 provided the optimum conditions to see differences in the proteins from tough and tender muscles. Several recent papers have also used SDS-PAGE to isolate certain characteristics of proteins. Labeit et al. (1990) conducted studies of titin and found that it contains 100 residue repeating motifs similar to other proteins which bind to myosin. Matsuura et al. (1991) used SDS-PAGE in conjunction with other techniques to isolate and identify the 1.2 million dalton subunit of titin and show that it is indeed elastic in

nature. Tan et al. (1993) found a repeating fibronectin type III pattern, and some unique differences between rabbit and chicken titin such as a serine rich region which may be a phosphorylation site. There have also been several recent studies using SDS-PAGE to observe the effects of added calcium to muscle proteins. Koohmaraie et al. (1988) infused carcasses with  $\text{CaCl}_2$  and noted an increase in proteolysis of several proteins and increases the band density of the 30,000 dalton component. Taylor and Etherington (1991) used SDS-PAGE methods to observe solubilization of proteins after adding a variety of solutions to the meat system, and Takahashi et al. (1992) employed SDS-PAGE techniques to note the splitting of the connectin (titin) molecule after calcium treatments. By the numbers of studies which involve the tracking of protein degradation, or purification with SDS-PAGE, it becomes apparent that this technique is essential in the understanding of protein functionality and structure, and will be a vital tool in understanding the relationship between protein degradation and tenderness.

### Water Holding Capacity

There are two main types of water in meat systems: bound and free. Bound water usually refers to the water which is water of hydration and tightly adhered to muscle proteins. Free water is the remaining water which is in the extracellular space, or is loosely bound with other charged molecules, and can be physically removed from the meat. The ability of meat to retain its moisture during and after handling is of extreme importance to the overall palatability of meat

cuts. If a muscle releases its fluids easily then it will have lower yields and be overly dry. Normal skeletal muscle contains approximately 70% water so it is vital to retain as much of this moisture as possible. The term which refers to a muscles ability to bind water is water holding capacity (WHC). WHC is critical for stored and processed meats, and is of considerable economic interest to meat producers, packers and consumers because meat is sold on a weight basis.

#### Factors affecting juiciness

There are a variety of factors which occur naturally, or that may be applied to postmortem muscle which will affect the WHC of various types of meat and meat products. The major factors which will be covered in this review include: pH, glycolytic rate, sarcomere length and diameter, temperature (freeze/thaw), salt/phosphates (removal of structural constraints), and divalent cations.

Miller et al. (1968) examined the pH effects on WHC and found that the WHC decreased as the pH neared the isoelectric point of the meat (approximately 5.5). Bouton et al. (1971) used pre-slaughter injections of epinephrine to create a wide pH range (5.6-7.0) in sheep. They observed an increase in juiciness as the pH increased, and when the meat homogenate was cooked at 65°C, cooking losses decreased as the pH increased. In a follow up study, Bouton et al. (1972) injected adrenaline pre-slaughter creating a pH range of 5.4 to 7.0. The researchers examined the effects of pH on moisture retention. They again found the same pH-juiciness relationship. Honikel et al. (1981a)



conducted studies to determine reasons for decreases in WHC in the postmortem period and concluded that less than one third of the total WHC decrease was due to a fall in pH. Hamm (1986) explained that a loosening of meat microstructure results in a increase in immobilized water due to the raising of the proteins net charge when either a acid or base is added. Furthermore, in the pH 5.0-6.5 range a pH change will have a considerable affect on the WHC due to ionization changes of histidine and glutamic acid residues; however, these changes are reversible. The WHC of meat from various animals depends on a number of factors other than pH, a number of which are, as yet, unidentified.

There may also be significant WHC changes in muscle due to temperature effects such as freezing. Muscle tissue water begins to freeze at  $-1^{\circ}\text{C}$ , and at  $-5^{\circ}\text{C}$  about 80% of water will be in a frozen state (Hamm, 1986). Freezing begins in the extracellular space, and as freezing progresses, the concentration of solids in the extracellular fluids increases, thus drawing water osmotically from within the cells. If the freezing process is rapid, the amount of cellular water moved to the extracellular space is minimized, and the ice crystals formed are smaller (Hamm, 1986). Miller et al. (1968) obtained conflicting results for the relationship between WHC and freezing depending on the method used to detect WHC. They found frozen meat had a higher WHC if a cooking WHC method was used; however, when a drip loss method was applied, the opposite results were found. Winger and Fennema (1976) used the amount of exudate as a WHC estimate and observed aged meat released no exudate upon freezing and thawing; however, unaged meat

did release a small amount of exudate. These results are in conflict with the report of Jakobsson and Bengtsson (1973) who found no exudate changes in longissimus samples frozen and then thawed four or 14 days postmortem. The differences between these studies may be due to storage temperatures. Winger and Fennema (1976) also found that the amount of exudate increased as the post-freezing storage time increased. Honikel et al. (1981a, 1981b) investigated the effects of postmortem storage in the 0°C to 30°C temperature range and observed no relationship between storage time and WHC. Due to the variability of results, it appears that there is some question as to the effect of freezing on WHC. Hamm (1986) suggests some possibilities for the observed variation in results. He suggests that the initial WHC may have a key role in the post-thaw WHC, and also the reabsorption capability of the muscle will affect the WHC. Other factors which can affect the WHC of frozen meat have been mentioned earlier in this review and include rate of freezing, rate of thawing and freezer storage.

Another type of temperature treatment which may effect the WHC is the application of heat. As the muscle is heat processed, the decrease in WHC appears to occur in phases. In the first phase, from 30°C to 50°C, changes are due to heat coagulation of actomyosin (Hamm, 1986). From 50 to 55°C, there are little if any WHC changes; however, from 60 to 90°C, denaturation of collagen occurs. Hearne et al. (1978) illustrated that heating rates played a key role in the cooking loss of beef, and Godsalve et al. (1977) concluded the WHC decrease of

meat during heating will ultimately determine the water loss rate. It is expected that the WHC is going to decrease with added heat, but the goal should be to minimize the moisture loss to obtain the most positive impact on palatability.

The addition of salts and/or phosphates has a large effect on various meat attributes, but the main advantage is an increase in the WHC. Sodium chloride (NaCl), commonly referred to as salt, causes a swelling of myofibrils and an increase in the WHC, making certain meat products are profitable to produce. The effect NaCl will have on WHC of meat depends on the pH. If the pH is above the isoelectric point (about 5.0 in the myofibrillar system), then NaCl will increase the WHC; however, if the pH is below the isoelectric point, then there will be a decrease in the pH (Hamm, 1986). Evidence indicates the effects of NaCl are due to the Cl<sup>-</sup> ions binding to positively charged amino acid groups on myosin and/or actomyosin. This association causes the isoelectric point to be shifted to a lower pH, and at a pH above this isoelectric point there will be a weakening of the bonds between opposite charged groups which allows room for swelling (Hamm, 1986). Offer and Trinick (1983) suggested, however, that the NaCl-induced swelling reaction is due to removal of structural constraints such as Z-lines, M-lines and myosin cross bridges as well as the electrostatic repulsion increase from the Cl<sup>-</sup> ions which all in turn increase the myofibrillar space to hold water. Paterson et al. (1988) observed the effects of NaCl (as well as phosphates, which will be mentioned later) on beef myofibrils, specifically observing the effects of swelling on

WHC. They found no significant myofibril swelling up to 0.4M NaCl, however at 0.7M and 1.0M NaCl there was a large degree of swelling. At 0.7M NaCl, the A-bands of myofibrils began to be extracted; however, the A-I region was resistant to extraction up to 1.0M NaCl. The WHC increased significantly as the NaCl concentration was raised from 0.1 to 1.0M. These researchers also used SDS-PAGE methods to observe that as titin degradation occurred, there was an increase in the WHC, leading to the conclusion that the removal of titin (gap filaments) allowed for an increase in myofibrillar swelling. Parsons and Knight (1990) also observed the effects of salt (and phosphate) on myofibrils; however, the main thrust of their work was the reason for the extraction of myosin. These researchers discovered the A-bands were extracted between muscle fibers rather than inside the myofibrils, and the extraction characteristics of myosin depends on the type of muscle fibers exposed to the NaCl solution. It appears that fast white fibers are extracted at low NaCl concentrations, and slow red fibers require higher concentrations. A recent study by Ockerman and Wu (1990) examined the effects of NaCl on cooking yields of an emulsion-type pork sausage. These researchers used hot-boned pork and added 2, or 3% NaCl. Initially the 3% samples had the highest WHC, but with increased chopping the WHC decreased to a equivalent of the 2% level.

In a number of studies the synergistic effect of phosphates in addition to salt were observed. Phosphates are used throughout the meat industry to improve the WHC of meat products. There are over 400 publications which involve the use of phosphates in meat products

(Hamm, 1986), which indicates its importance to the meat industry. Hellendorn (1962) found that polyphosphates will not greatly increase the WHC by themselves; however, in conjunction with NaCl, there was an increase in the WHC. Shults et al. (1972) observed the effects of various phosphates on the WHC of beef muscle. It was found that polyphosphate and tripolyphosphate had the greatest effect on meat swelling, and it appeared the pH change which occurred when the phosphates were added caused the observed increase in WHC. Offer and Trinick (1983) observed a cooperative action between salt and phosphate. In their study, it was found that pyrophosphate greatly reduced the amount of NaCl needed for maximal swelling to occur. In addition, when phosphates were used, the entire A-band was extracted rather than just its middle portion. Trout and Schmidt (1983) suggested several mechanisms by which phosphates may enhance the WHC. They found that phosphates increase the pH and ionic strength, dissociate the actin-myosin complex, and chelate divalent metal ions. Young and Lyon (1986) marinated chicken breast meat in tripolyphosphate and found the phosphate samples had greater cooked yield, and also absorbed the most marinade when compared to a control. It was pointed out by Hamm (1986) that phosphate, by itself, exerts little if any effect on the WHC since there is no electrostatic repulsion between protein molecules. Offer and Trinick (1983) and Paterson and Parrish (1988) found that the addition of pyrophosphate to salted meat systems would decrease the NaCl requirement needed for maximal swelling, increase the amount of protein extraction, and significantly

improve the WHC. Dziezak (1990) provided a comprehensive review of phosphates used in the food industry, and pointed out that in meat products, phosphates serve to form a chelating complex with magnesium and calcium (divalent cations) which then allows actomyosin dissociation.

There has been some research investigating the effect of divalent cations on WHC. Regenstein and Rank Stamm (1979a; 1979b) conducted two studies observing the effects of  $\text{CaCl}_2$ , and  $\text{MgCl}_2$  in addition to phosphates on the WHC of muscle from various species. It was concluded that in all the animal tissue tested (rainbow trout, lobster tail, and chicken breast), the divalent cations either had no positive effect, or in most cases had a negative effect on the WHC due to a repulsion of negative charges. Hamm (1986) explains that divalent cations are bound to myofibrils in three ways: 1) pH dependent bonds which are strong at pH 7.0 and weaken as the pH decreases, 2) myosin isoelectric point (pH 5.0) stable bonds, 3) electrostatic binding to negatively charged protein groups. It was further explained that divalent cations will decrease the WHC in meat products because the cation binding reduced the repulsion between the negatively charged groups, which causes the protein structure to tighten and allow less area for water to be held.

Another factor which may affect the WHC is the glycolytic rate of the postmortem muscle. The pH-WHC relationship was explained earlier in this review, however there are a few more areas which should be covered. Several WHC studies have used pale, soft and

exudative (PSE) pork as well as watery, pale beef due to their unique glycolytic rates. In both beef and pork muscles, the decrease in the WHC of these animals was mainly due to a rapid postmortem degradation of glycogen and ATP (Fisher and Hamm, 1980; Hamm, 1986). The condition thought of as opposite to the PSE condition in pork is dark, firm, and dry (DFD) meat (termed dark cutter in beef). This meat has low amounts of glycogen in the early postmortem period, and high WHC values due to a higher pH. Due to the high WHC of this meat, it is beneficial to use the DFD product in emulsified type products (Hamm, 1986).

There are a number of other factors which can influence a muscles WHC. One of these factors is hot-boning (the removal of muscles prior to rigor onset), which is sometimes used as a source of meat for emulsified products. A decrease in the WHC of hot-boned meat occurs in conventional cutting systems; however, if the hot-boned meat is slow chilled, there will be little if any differences in the WHC (Kastner et al., 1973; Hamm, 1986) when compared to conventional processing. The effect of sarcomere length on the WHC of meat has been investigated and a linear relationship between sarcomere shortening and drip loss depends on the contractile state of the meat after the onset of rigor has been reported (Honikel et al., 1986). Offer and Trinick hypothesized that water losses in meat was due to a shrinkage of the filament lattice. Rao et al. (1989) measured the WHC of six beef muscles, and used light microscopy to detect sarcomere lengths and diameters of those muscles. These researchers found that

the diameter of the muscle fiber was of greater importance to the swelling effect (increased WHC) than sarcomere length. The WHC of a meat system can also be altered by the addition of water binding compounds (starches, gums).

Today, due to the public's demand for a leaner meat product, many meat processors are using binders and/or extenders in an attempt to create low fat products which are still of high quality. Foegeding and Ramsey (1986, 1987) conducted two studies which observed the interaction of several gums in meat batters. These researchers tested carrageenans and methylcellulose in low-fat, high-moisture meat batters and found that the carrageenans were the most beneficial in binding added water. They concluded the use of these gum products allows for the replacement of fat with no detrimental effects in WHC or palatability; however, each gum has its own benefits and disadvantages which suggests use of gum combinations would achieve optimal results. Brown and Zayas (1990) looked into extending beef patties with corn germ protein flour. They found that the addition of this protein would increase yields by enhancing the WHC. This is a rapidly growing field of meat science and will probably keep growing due to consumer demands, and possibility, of processing profitability. Recently, the irradiation process has begun to be accepted by U.S. consumers in order to make meat and fruit/vegetable and poultry products more wholesome. Ionizing radiation has been shown to have little if any detrimental effect on the WHC of meat product, or any other quality attribute for that matter (Lawrie et al., 1961; Radomyski,



1994). A number of factors which affect the WHC have been discussed, and in many situations several, of these factors can be manipulated to improve the WHC.

#### Measurement techniques

There has never been a universally accepted way of measuring WHC. It seems a number of labs each has their own way for estimating the WHC. There are centrifugation, press, drip loss, cooking and suction methods to determine the loosely bound water which is used to estimate WHC. One method which is accepted to a higher degree than most others is the hydraulic press method. Its acceptance is mainly due to its longevity of use, simplicity and familiarity of methodology by researchers. The first hydraulic press method (filter-paper press method) was developed by Grau and Hamm (1953); however, it has been modified a number of times before reaching the method used today. For this method 0.3 grams of muscle is placed on a sheet of filter paper (Whatman #1) and then placed between two plexiglass sheets. The plates are placed in a hydraulic press and exposed to 3000 psi for three minutes. The filter paper is then removed from the plexiglass plates and both the water outline (total area) and meat outline are traced. A planimeter is used to measure the areas of the outlines created. The actual WHC estimate reported may vary, some labs report the total area divided by the meat area, while other labs may report the area of the water ring (total area minus the meat area). The percent free water (total area minus meat area multiplied by 61.1 and then divided by the

total water content), or the ratio of muscle area to total area may also be reported.

Centrifugation is another common method for estimating WHC; however, numerous variations are available to researchers. A main difference of the methods is the use of high speed centrifugation versus low speed centrifugation. High speed centrifugation techniques use speeds into the 100,000's x g to separate out components without heating them. Bouton et al. (1971) used centrifugation speeds of 120,000 to 190,000 x g for 30 to 60 minutes with no added water, and concluded that this technique has a number of advantages including its flexibility for testing raw or cooked meats. Other advantages include the acquisition of juice which can be further analyzed, and easily reproducible results. Bouton et al. (1972) modified their previous procedure by centrifuging meat samples at 100,000 x g for one hour and obtained more consistent results; however, this method requires a high speed centrifuge, and it does not correlate well with other WHC methods (Hamm, 1986). Lower speed centrifugation techniques have historically been the more popular type of centrifugal methods due to their repeatability and widespread use. Wierbicki et al. (1957b) described a method for determining WHC in which meat was placed on fritted glass within a centrifuge tube. After centrifugation at 12,000 x g the amount of water which had been extracted was measured. This method was modified by Miller et al. (1968) when they used tubes with built in holes covered by filter paper, and used 20 grams of meat centrifuged at 12,000 x g. Dagbjartsson and Solberg (1972) further

modified the procedure by using a molecular sieve placed into a centrifuge tube and covered with filter paper. One gram of pulverized meat was added to the tube, and after centrifugation at 12,000 x g, the water lost was measured. These researchers concluded that this was a reliable and repeatable method for the determination of WHC. A different type of centrifugal method was applied by Regenstein et al. (1979) who used a 1:20 meat to phosphate buffer solution ratio which was centrifuged at 31,000 x g. After centrifugation, the supernatant was decanted, the inside of the tube dried and a final weight collected for a WHC estimate. A value was determined by dividing the grams of water in the pellet by the grams of protein in the pellet. These researchers were convinced that this WHC method provided as reliable, or more reliable results than other methods available. Katoh (1981), using parts of several different methods, placed tissue paper in the bottom of a centrifuge tube topped by one gram of fresh muscle (minced) and centrifuged at 671 x g for four minutes. The sediment was then analyzed for moisture content, and this value was used as the WHC estimate. These researchers concluded that this method gave precise results, required only standard laboratory equipment, and required a small amount of sample. Jauregui et al. (1981) developed a simple method for determining the expressible juice in a meat sample by collecting the water released using filter paper when meat was exposed to 7,710 to 30,900 x g centrifugal force. These researchers believed this method was very repeatable and accurate. The main disadvantages of low speed centrifugation were the need for specially

made centrifuge tubes; these methods were not rapid, were more complex and some microstructural changes were produced (Trout, 1988).

There are two main methods categorized as capillary action, or suction techniques. Hofmann, (1975) used a gypsum block procedure, termed the capillary volumeter method. In this method, 50 grams or more of muscle sample was pressed between a porous gypsum plate and a steel plate for about 60 seconds. The gypsum absorbs the released water, which displaces water in the plate. This air is then displaced and measured as the WHC estimate. This method is quite rapid, and reproducible, however the gypsum plate porosity creates a major source of variability since it can not be controlled. A second method was described by Kauffman et al. (1986) which used filter paper placed directly on a freshly cut meat surface for two seconds, and then obtained the weight differential of the wet and dry filter paper. This method is extremely rapid, simple, and requires no special equipment; however, the amount of time which the paper is left on, the pressure which is used to place the filter paper on the meat surface, and humidity conditions will affect the results of this method (Beekman and Parrish, 1993).

There are a variety of cook yield WHC methods available to researchers. These methods allow WHC estimates to be achieved without the use of a centrifuge by weighing cooked ground, or unground, meat samples. Sanderson and Vail (1963) used a procedure termed tube cooking, in which a 2 inch by 3/4 inch strip of muscle with an inserted thermometer was wrapped in foil and placed in a centrifuge tube. The

tube contents were heated in a water bath to an internal temperature of 140 to 176°F. The meat was allowed to cool in the foil to 104°F, removed from the foil, and then rolled onto paper towels prior to being weighed which yielded the WHC estimate. Honikel et al. (1981a) added five grams of muscle sample to a preweighed centrifuge tube, covered by a glass marble, and placed in boiling water for 20 minutes. The tube was cooled and the excess juice was poured off. Then the meat was removed from the tube, blotted with filter paper prior to being placed back into the tube, and reweighed. These researchers also used this procedure in a companion study (Honikel et al., 1981b) to achieve WHC estimates. Whiting and Miller (1984) applied a procedure where meat samples were homogenized, placed into a container for cooking, cooked to an internal temperature of 71°C, cooled and weighed for the cooked differential. One step that is critical for all cook yield methods is the removal of the meat from the cook-out juices in order to prevent any fluid reabsorption. These methods have several advantages including the use of a large representative sample, and the relative speed of the procedure (20 to 30 minutes). The main advantage of the cook-loss procedures is the ability to predict large scale cook yields even when salts, phosphates, and homogenization steps are used (Trout, 1988). These methods do have disadvantages including high start up costs, the method is not as rapid as some other techniques and only cooked products can be tested.

The estimation of WHC using drip loss methodology was tested, but failed to become popular in the majority of laboratories. Honikel et

al. (1980) proposed a method in which a 30 gram cube of meat was sealed in a plastic bag and stored such that the meat never touched the bag sides or the released drip. The WHC estimate (drip loss) was then calculated by a weight differential. Another method which is not used to a great extent is nuclear magnetic resonance (NMR). WHC is measured indirectly by the measuring of the pore sizes of areas holding immobilized water. This method is quite accurate, however cost per sample, and procedural complexity are major disadvantages. Some researchers use a combination of methods. For example, Paterson et al. (1988) used a combination of cook loss and low speed centrifugation methodology in obtaining their WHC estimates and acquired quite consistent results.

Due to the number and variety of WHC methods available, it is difficult to recommend one method unequivocally for every situation. It appears that much of the decision on which method to use is determined by the type of material, funding capabilities, time allowances, and personnel staffing.

#### Relationship between WHC and tenderness

It has been documented by several researchers that the degree of hydration and the amount of water binding of a given sample will affect tenderness (Arnold et al., 1956; Wierbicki et al., 1956; Deatherage, 1957; Hamm, 1959). The previous studies were only able to speculate on the nature of tenderness and WHC, but McClain and Mullins (1969) conducted a study to observe the relationship between tenderness and a variety of attributes including WHC. They found that differences in

WHC could not explain the variation in tenderness which existed. Bouton et al. (1971) found that tenderness changes appeared to be related to changes in pH which were also in line with WHC variations; however, no direct linkages could be made. Young and Lyon (1986) found that chicken breast meat treated with phosphate had a higher WHC as well as an increase in tenderness when compared with controls and treated groups using  $\text{CaCl}_2$ , or NaCl. From the research conducted, there seems to be no direct relationship between WHC and tenderness; however, the WHC of meat is usually indirectly linked to tenderness by consumers because a juicy cut of meat is perceived as more tender, therefore it is difficult for the majority of consumers to separate the two attributes of juiciness and tenderness.

#### Effects of injection on WHC

Injection of solutions into meat cuts have been used for a number of years in an attempt to enhance various aspects of fresh meat. The effect of adding salt and/or phosphates to meat systems and the beneficial WHC results which occur have been previously mentioned. Wheeler et al. (1991) injected 0.3M solutions of  $\text{CaCl}_2$  and observed an increase in the cooking losses of both bull and steer biceps femoris muscle; however, the semimembranosus muscle cooking loss was not altered by the  $\text{CaCl}_2$  treatment. Morgan et al. (1991a) found no enhancement of WHC (juiciness) when  $\text{CaCl}_2$  treatments were injected into cow steaks, and Wheeler et al. (1993) saw enhanced WHC of longissimus muscle only when compared to a non-injected control. However, Wheeler et al. (1992) saw decreased cook loss when meat was

injected at 30 minutes postmortem with 0.3 M  $\text{CaCl}_2$ , and increased cook loss when injected at 24 hour postmortem. Results seem to lack uniformity when the effect of  $\text{CaCl}_2$  on the WHC of meat is addressed.



PAPER: THE EFFECTS OF CALCIUM CHLORIDE, TRIPOLYPHOSPHATE  
AND SODIUM CHLORIDE INJECTIONS ON THE PALATABILITY,  
WATER HOLDING CAPACITY AND MYOFIBRIL PROTEIN  
DEGRADATION OF BEEF LOIN MUSCLE

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## ABSTRACT

Bovine longissimus lumborum muscle was converted into loin steaks at 24 hours postmortem and injected to a 10% weight basis with water, and solutions of sodium chloride, tripolyphosphate and calcium chloride. Steaks were postmortem aged 3, 6 and 13 days postmortem and the effects of the injection treatments and postmortem aging times were analyzed for: sensory attributes, including tenderness and bitterness, water holding capacity (WHC), cook yield, myofibril fragmentation index, and myofibrillar protein degradation.

NaCl/phosphate and NaCl/phosphate/CaCl<sub>2</sub>-treated steaks had significantly lower Warner-Bratzler shear values than the control at 3 days postmortem. These steaks also had the highest sensory tenderness scores, and the most improved WHC values at 3, 6 and 13 days postmortem. CaCl<sub>2</sub>-treated steaks, although more tender than the controls, had the highest bitterness scores. Furthermore, CaCl<sub>2</sub> steaks had accelerated titin degradation, earlier 30,000 dalton component appearance, and higher MFI values indicating the mechanism of tenderization by CaCl<sub>2</sub> was proteolysis. Our results suggests NaCl/phosphate injection treatment would offer more advantages to the beef industry than CaCl<sub>2</sub> treatments.

## INTRODUCTION

The need to develop a consistently tender beef product has recently become a top priority for producers and processors due to consumer driven demands. The reason one animal produces tender steaks while another produces tough steaks has perplexed the meat industry for many years. It seems that tenderness is known to be affected by a large number of independent factors. Because of these ante- and postmortem factors, the meat/muscle system is one of the most complex systems in nature. Consequently, achieving uniform tenderness is a challenging problem requiring innovative approaches.

Many methods have been studied in an attempt to enhance beef tenderness. These methods include: blade tenderization (Booren et al., 1981; Rolan et al., 1988; Benito-Delgado et al., 1994), natural postmortem aging (Parrish et al., 1973; Marsh, 1983; Huff and Parrish, 1993), chilling rates (Davey and Graahuis, 1976; Honikel et al., 1986), electrical stimulation (Savell, 1979; Bouton et al., 1980; McKeith et al., 1981), pH (Marsh et al., 1987; Pike et al., 1993), enzymatic reactions (Dutson, 1983; Zeece et al., 1986; Goll et al., 1992; Koohmaraie, 1992; Dransfield, 1993), and chemical treatments (salts, phosphates) (Shults et al., 1972; Offer and Trinick, 1983; Koohmaraie et al., 1988; Paterson et al., 1988; Wheeler et al., 1993).

The effect of injecting compounds into muscle on certain proteins composing the myofibril can be systematically studied by using SDS-PAGE and microscopy techniques. Successful use of SDS-PAGE has been accomplished by several researchers who discovered the appearance of

a 30,000 dalton band and titin degradation earlier in tender steaks (MacBride and Parrish, 1977; Olson et al., 1977; Anderson and Parrish, 1989; Huff Lonergan et al., 1994b).

Recently, it has been shown that injection of beef loins with solutions of calcium chloride ( $\text{CaCl}_2$ ) resulted in rapid tenderization (Morgan et al. 1991; Wheeler et al., 1991; Tatsumi and Takahashi, 1992; Wheeler et al., 1993). Infusion of carcasses and marination of retail cuts with  $\text{CaCl}_2$  to tenderize the cuts have also been used (Koochmaraie et al., 1988; Whipple and Koochmaraie, 1993). It has been speculated that the addition of  $\text{CaCl}_2$  to the muscle system will enhance the action of the calpain enzyme system (Whipple and Koochmaraie (1993). Results of the addition of  $\text{CaCl}_2$  to meat systems has shown a significant increase in the tenderness of treated meat samples; however, at higher concentrations some bitterness off-flavoring was noted (Morgan et al., 1991; Benito-Delgado et al., 1994). 20% of the population can detect bitterness (Harris and Kalmus, 1949; Glanville and Kaplan, 1965; Bartoshuk, 1979), which makes it important to note which, if any, panelists are sensitive to bitterness sensations when conducting sensory panels with  $\text{CaCl}_2$  treatments. If one fifth of the population is able to detect an objectionable flavor in a product due to  $\text{CaCl}_2$ , careful considerations must be given before promoting its use in the commercial market.

Several researchers have added salts and phosphates to meat systems and found an increase in the ionic strength, and therefore, an increase in the water holding capacity (Trout and Schmidt, 1983;

Paterson et al., 1988). Offer and Trinick (1983) noted a degradation of the Z-line, and M-line, weakening of actomyosin crossbridges, and Paterson et al. (1988) observed increased solubilization of titin and nebulin when salt and phosphates were added to meat. Removal of these structural constraints enhances tenderness and WHC of treated muscles.

The objectives of this study were to determine the effects of injecting solutions of calcium chloride, sodium chloride, tripolyphosphate, and a combination of these compounds into *longissimus dorsi* steaks from A maturity carcasses on: 1) palatability attributes, including tenderness (WB shear and sensory tenderness), juiciness, bitterness and saltiness, 2) WHC, and 3) degradation of myofibrillar proteins as monitored by SDS-PAGE and indirect immunofluorescence.

## **MATERIALS and METHODS**

### **Sources, injection and storage of muscle**

Five A maturity Choice/Select market steers were processed at the Iowa State University Meat Laboratory following standard slaughter procedures. Carcasses were not electrically stimulated. Loins (longissimus dorsi) were excised from carcasses stored in a 2°C cooler for 24 hours postmortem. The loins were immediately cut into 24 1.9 cm steaks, and injected to a 10% (w/w) level with one of four solutions to give a final concentration in the muscle as follows: 1) distilled water control, 2) 0.75% sodium chloride (NaCl)/0.35% tripolyphosphate, 3) 0.15% calcium chloride (CaCl<sub>2</sub>), 4) 0.75% NaCl/0.35% tripolyphosphate/0.15% CaCl<sub>2</sub>. The injection apparatus consisted of four 3 cc syringes fastened to each other creating an injection width of 6.35 cm. For a 10% injection level to be achieved, the steaks were manually injected every 1.27 cm. Steaks and any excess liquid were vacuum packaged and stored three, six, or 13 days postmortem in a 2°C cooler prior to being used for further analysis, or frozen for subsequent sensory analysis. After the postmortem aging period, a thin slice was removed from the surface of all the steaks. These thin slices were collected for further analysis prior to the steaks being cooked for Warner-Bratzler shear analysis.

### **Sensory analysis**

Analytical sensory panels were composed of eight members trained for the attributes to be tested (juiciness, softness to tooth

pressure, fiber fragmentation, saltiness and bitterness). Panelists were required to attend two training sessions of one hour each. During these sessions panelists were presented with samples and asked to describe textures and/or flavors of beef samples. The panelists were trained for evaluating juiciness by sampling beef steaks that had been cooked to different end point temperatures (59°C versus 71°C). Care was taken to restrict panelist opinions to the single attribute being examined, and it was emphasized that likes and/or dislikes must have no effect on the panelists scores. Softness to tooth pressure and fiber fragmentation were explained to, and tested by the panelists during training sessions comparing round steak with tenderloin steak samples. Saltiness was described and analyzed by the panelists using loin steaks injected with selected NaCl solutions. Bitterness was described as the sensory perception associated with caffeine and high concentrations of  $\text{CaCl}_2$ . Panelists were trained for the bitterness attribute using various concentrations of  $\text{CaCl}_2$  ( 0.05M, 0.15M, 0.3M, 0.4M).

Sensory steaks that had been frozen after the desired aging period were thawed for 16 hours at 2°C before being placed onto broiler trays and were thermally processed in a General Electric model CN02 industrial broiler (Chicago Hts., IL), 10.16 cm from the heat source with an internal oven temperature of 288°C. Steak temperatures were monitored with Teflon-coated copper thermocouples (Omega, Stamford, CT) attached to an Omega 406 power pak recorder (Stamford, CT). A hand held Taylor model 9800 K type thermocouple was also used to monitor cooking temperatures. The steaks were turned when they

reached an internal temperature of 24°C, and removed from the heat source at 65°C. Two representative samples (1 cm cubes) per steak were served to the panelists. Each panel session consisted of six different steak samples (treatments, one, two, three and four of either three or six day aged samples, and treatments one and four, or two and three of the 13 day aged samples), with individual samples being served four minutes apart. The scores were recorded by each panelist on a 15 cm line with anchor points 1 cm from each end. Every sample was assigned a random three digit number. Panelists were placed in partitioned cubicles with red lighting overhead to minimize biased responses. The panelists were instructed to cleanse their palates between samples with salt free crackers and room temperature water.

### **Shear force determination**

Steaks were subjected to cooking by being placed onto labeled broiler pans which were modified to avoid the mixing of steak fluids. Steaks were cooked in the same manner as for the Warner-Bratzler shear steaks. The steaks were cooled to room temperature (20°C) prior to the removal of six, 1.27 cm cores per steak by hand, with two cores each from the medial, lateral and central portions of the steaks. Cores were removed parallel to the fiber direction, and were sheared through the middle of the core by using an Instron Universal Testing Device model 4502, with a model 4500 computer assist module (Canton, MA). A Warner-Bratzler shear device was attached to the Instron, and the six cores were sheared perpendicular to the fiber direction, being



careful to avoid obvious connective tissue. Peak values (myofibrillar peak) were the data points collected and reported.

### **Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)**

Purified myofibrils were prepared for SDS-PAGE analysis. For preparation of myofibrils, a modified procedure of Wang (1982) was followed. A minimum of five grams of minced tissue was weighed and suspended in 10 volumes (50 mL, v/w) of standard salt solution (SSS: 100mM KCl, 20mM K phosphate, 2mM MgCl<sub>2</sub>, 1mM EGTA, 1mM NaN<sub>3</sub>). This was homogenized for 10 seconds in a Waring blender (New Hartford, CT), and centrifuged (Beckman Instruments model J2HS, Palo Alto, CA) for 10 minutes at 1000 x g. The pellet was resuspended in six volumes of SSS, homogenized for 10 seconds, and centrifuged for 10 minutes at 1000 x g. Next pellets were resuspended in eight volumes of SSS, homogenized for 10 seconds, and centrifuged at 1000 x g for 10 minutes. Pellets were again resuspended in eight volumes of SSS, strained through a nylon mesh strainer with the aid of a stir rod, and centrifuged at 1000 x g for 10 minutes. The pellet was resuspended in six volumes of SSS and 1% Triton X-100, homogenized for 10 seconds, and centrifuged for 10 minutes at 1500 x g. The previous step was repeated once the pellets were resuspended in eight volumes of SSS and centrifuged at 1500 x g for 10 minutes. The pellet was resuspended in eight volumes of KCl (100mM) and centrifuged for 10 minutes at 1000 x g. The previous step was repeated and the pellet resuspended in 10

volumes of Tris-HCl, pH 8.0 wash buffer and centrifuged at 3020 x g for 10 minutes. This step was repeated once to obtain the final myofibril sample. Protein concentration was determined using the modified biuret procedure (Robson et al., 1968).

For SDS-PAGE analysis, isolated myofibril suspensions were combined with 0.5 volume tracking dye (30mM Tris-HCl pH 8.0, 3mM EDTA, 3% SDS, 30% glycerol and 0.001% pyronin Y) and 1/10 volume B-mercaptoethanol prior to being heated at 50°C for 20 minutes. The myofibril preparation (2ug/ul) was loaded onto each lane (25ul total) of a Hoefer gradient gel apparatus (San Francisco, CA). Three to twelve (acrylamide: bisacrylamide=100:1), and twelve to twenty percent (acrylamide: bisacrylamide=37:1) (5% stack, acrylamide:bisacrylamide=100:1) acrylamide gradient gels were made. A ten well comb was used in the gradient gels allowing the following to be loaded on one gel: three postmortem age group controls, three treatments within one postmortem age group, a zero day sample, a purified bovine titin standard, a purified bovine nebulin standard, and a high molecular weight standard. The three to twelve percent gels were run for 18 hours at six milliamps, and the twelve to twenty percent gels were run for 14 hours at 10 milliamps. The gels stained for six hours in 0.1% Coomassie Brilliant Blue solution (40% ethanol, 7% acetic acid).

**Indirect immunofluorescence**

The isolated myofibrils prepared for SDS-PAGE were also analyzed by dual label indirect immunofluorescence for titin detection. Six to eight drops of glycerinated myofibrils were placed on glass coverslips that were sitting on parafilm covering damp paper towels, and allowed to incubate at room temperature for five minutes. The myofibril solution was suctioned off and the coverslips were bathed three times in a buffer (75mM KCl, 2mM MgCl<sub>2</sub>, 2mM EGTA, 20mM Hepes, pH 7.6) for two minutes each time. The last buffer bath was suctioned off, and the coverslips were inverted onto a 0.50 uL drop of the primary antibodies being used for analysis. The primary monoclonal anti-titin was produced by our lab, bound in the I-band area, and was labeled 4C7. The primary polyclonal anti-desmin was also produced in our lab, and serves to label the Z-line. After a two hour incubation at room temperature, the coverslips were turned over and bathed in buffer for one minute. The bathing was repeated three times. A 0.50 uL drop of the secondary antibodies (Titin: Sigma F-4018 Goat anti-mouse IgG, desmin: Sigma T-6778 Goat anti-rabbit IgG TRITC, Sigma, St. Louis MO), diluted 1:100 in buffer, was placed on the parafilm, and the coverslips were inverted onto it and incubated for 30 minutes at room temperature. Coverslips were turned over and bathed in buffer for five minutes. The bathing was repeated three times. The buffer was suctioned off and the coverslips were placed onto glass slides containing 5.0 uL of mounting medium (FITC Guard, Testog, Inc., Chicago IL). Any excess medium was removed with Kimwipes, and the

coverslips were sealed with clear fingernail polish. Slides were viewed with a Noran Instruments Odyssey Confocal microscope illuminated with an argon laser. Negatives were obtained using Kodak Technical Pan 2415 film developed in 100% Dektol.

### **Myofibril fragmentation index**

The short method of myofibril fragmentation index (MFI) was determined using the procedure of de Pulgar (1982). All the procedural steps with the exception of the spectrophotometric reading were carried out in a cold room (2°C). Briefly, four grams of minced muscle was combined with 40mL of an isolating medium (100 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM NaN<sub>3</sub>, 20 mM potassium phosphate buffer pH 6.85) in a Waring blender. After homogenizing for 30 seconds the homogenate was strained through a polyethylene strainer. A 0.25 mL sample of the vortexed homogenate was diluted by the addition of 9.75 mL of isolating medium. The spectrophotometer was blanked by using isolating medium. Absorbances of samples were read at 540 nm after vortexing for 5 seconds. MFI values were then obtained by multiplying the absorbance by 200.

### **Water holding capacity**

A hydraulic press method was employed for the determination of water holding capacity (WHC) (Grau and Hamm, 1953). For this method, 0.3 grams of muscle was placed onto a piece of dried #1 Whatman filter paper (prepared in triplicate). The sample and filter paper were

placed between two plexiglass plates, and 3000 psi (Carver Laboratory Press, Summit, NJ) was applied for three minutes. The plates were removed from the press and the meat and water areas were traced with a number 2 pencil prior to being removed from the plexiglass plates. The traced areas were measured using a compensating polar planimeter, and the water area was divided by the meat area for a WHC value.

### **Cook yield**

Weights of the steaks were recorded at selected times during the experimental procedure. An initial weight was obtained to determine the amount of solution to be injected. A second weight was collected after samples were obtained, and a third weight was acquired just prior to cooking. The final weight was collected after the steaks were cooked. For estimating cook loss, the post-cooked weight was divided by the post-sampling, injected, precooked steak weight.

### **Statistical evaluation**

Data were analyzed by ANOVA with the GLM, CORR and MEANS procedures of SAS (1988) for a split-plot design. The whole plot was injection treatment and the split-plot was postmortem aging time. Contrast statements were written to test for significant treatment differences.

## RESULTS and DISCUSSION

Data from sensory panel evaluation of control and treated loin steaks are shown in Tables 1 to 3. Table 1 contains the sensory means of control injected and treated steaks aged 3 days postmortem. NaCl/phosphate injected steaks had significantly higher values ( $P < .05$ ) for softness to tooth pressure, fiber fragmentation, juiciness and saltiness compared with the control and  $\text{CaCl}_2$  samples aged 3 days postmortem. Softness to tooth pressure and fiber fragmentation scores from Table 1 indicate a large tenderizing effect in the NaCl/phosphate-treated groups, which also had the highest juiciness and saltiness values at this postmortem aging period.  $\text{CaCl}_2$ -treated steaks had significantly higher ( $P < .05$ ) bitterness values compared to the other injection treatments. Our results agree with those of St. Angelo et al. (1991), Benito-Delgado et al. (1994) and Morgan et al. (1991) who all noted a bitterness sensation associated with  $\text{CaCl}_2$  addition. In a very detailed sensory analysis of lamb carcasses infused with a 0.3M  $\text{CaCl}_2$  solution, St. Angelo et al. (1991) found a significant difference ( $P < .01$ ) in bitterness between non- $\text{CaCl}_2$  infused and infused carcasses. Benito-Delgado et al. (1994) injected a 0.3M  $\text{CaCl}_2$  solution into infraspinatus muscles and sensory panelists observed a lower flavor scores for the injected steaks. Morgan et al. (1991) found panelists characterized  $\text{CaCl}_2$  injected strip loins as being more bitter in taste. We disagree; however, with the interpretation of results from St. Angelo et al. (1991) by Wheeler et al., (1991) who reported only

slight bitterness problems occur with infusion of  $\text{CaCl}_2$  into lamb carcasses.

Table 2 contains sensory results of the 6 day postmortem control and injected steaks. This data showed that for softness to tooth pressure and fiber fragmentation, the three groups of treated steaks were significantly more tender ( $P < .05$ ) than the control steaks. No significant difference for sensory tenderness was observed among the three treatments ( $P > .05$ ). Although it was not significant, the NaCl/phosphate containing treatments again had the highest sensory values for sensory tenderness. Also, the NaCl/phosphate-treated steaks were significantly ( $P < .05$ ) more juicy than the control steaks, but not different from the other treatments. Again the  $\text{CaCl}_2$ -treated steaks had the highest value for bitterness and were significantly more bitter ( $P < .05$ ) than the control. The NaCl containing treatments had significantly higher saltiness values ( $P < .05$ ), and the NaCl/phosphate/ $\text{CaCl}_2$  treatment was significantly more salty than the NaCl/phosphate treatment. It should be mentioned that the NaCl level used (0.75%) is quite low when compared with other processed meat products.

The data from the 13 day postmortem aged control and injected samples are presented in Table 3. For softness to tooth pressure and fiber fragmentation, NaCl/phosphate/ $\text{CaCl}_2$ -treated samples again exhibited the highest values, but were not significantly different from the NaCl/phosphate samples. The NaCl/phosphate/ $\text{CaCl}_2$ -treated steaks were also significantly more juicy than the water control steaks which

would indicate a higher WHC. Not surprisingly, the same saltiness trend was present in the 13 day samples as was present in the 3 and 6 day samples in that the NaCl/phosphate, and NaCl/phosphate/CaCl<sub>2</sub> treatments had significantly higher ( $P < .05$ ) saltiness values compared to the other injected samples. The CaCl<sub>2</sub> treatment exhibited the highest bitterness values which were significantly different ( $P < .05$ ) from the control and the NaCl/phosphate/CaCl<sub>2</sub>-treated steaks. In this study, three of the eight panelists were able to detect bitterness, while the other five were not able to detect it. When the data from panelists able to detect bitterness were excluded from the analysis and the means recalculated, no significant differences were detectable for the bitterness attribute in CaCl<sub>2</sub>-injected steaks. Therefore, the number of panelists able to detect bitterness should be identified if bitterness is to be tested. It seems that bitterness would be a concern in CaCl<sub>2</sub> treated steaks, which is in agreement with a number of studies (St. Angelo et al., 1991; Benito-Delgado et al., 1994; Morgan et al., 1991). Although our results indicate and reinforce that a beef product containing CaCl<sub>2</sub> is more tender, injection of CaCl<sub>2</sub> should be carefully considered before introducing a product to the market because 20 percent of consumers can detect bitterness (Glanville and Kaplan, 1965; Bartoshuk, 1979). The palatability traits equilibrated over time, and by 13 days postmortem there was little to no differences in the softness to tooth pressure, fiber fragmentation and juiciness values. This suggests that the injection treatments used



were most beneficial, when compared to the control, in steaks aged 3 and 6 days postmortem.

The results from WB shear analysis are presented in Tables 4, 5 and 6. The WB shear means illustrate that the three treatments were significantly ( $P < .05$ ) more tender than the controls for the three postmortem aging periods, but no differences existed among the three groups of treated samples. Although there was a decrease in the WB shear values of  $\text{CaCl}_2$ -treated steaks we did not observe as large a WB shear decrease as several studies which infused or injected  $\text{CaCl}_2$  into beef longissimus, and biceps femoris muscles (Morgan et al., 1991; Wheeler et al., 1991; Wheeler et al., 1993). However, a recent study by Benito-Delgado et al. (1994) which examined 0.3M  $\text{CaCl}_2$  injections of beef longissimus and infraspinatus muscles, failed to observe any decrease in WB shear values from steaks injected with  $\text{CaCl}_2$ . It is interesting to note that the NaCl containing treatments had the lowest WB shear force values. A certain degree of WB shear improvement was expected with NaCl injection due to the solubilization of proteins; however, in our study the NaCl/phosphate and NaCl/phosphate/ $\text{CaCl}_2$  treatments had lower WB shear values, and higher sensory tenderness values over all the aging periods. Given that NaCl and phosphates increase WHC (Offer and Trinick, 1983) our results suggest that muscle protein hydration may be equally, or more important in enhancing muscle tenderness than addition of calcium which stimulates proteolysis by calpains. It seems the application of NaCl and phosphate treated steaks would be a viable method of tenderization for steaks

without introducing bitterness to the consumer market since this is already a commonly used practice in processed meat manufacture.

Figure 1 contains the SDS-PAGE results of the 3–12% gels of myofibrils isolated from the  $\text{CaCl}_2$ -treated steaks for all the aging periods. From the analysis of the gel, it is evident that there are both T<sub>1</sub> (upper band) and T<sub>2</sub> (lower band) forms of titin in the control aged 3 days postmortem; however, the myofibrils isolated from the  $\text{CaCl}_2$ -treated steaks have only T<sub>2</sub> at 3 days postmortem. This is an original finding as this is the first time a banding pattern from  $\text{CaCl}_2$ -treated steaks demonstrating the transformation of T<sub>1</sub> to T<sub>2</sub> has been reported. It should be noted that T<sub>2</sub> has been shown to be a degradation product of T<sub>1</sub> (Wang et al., 1979; Huff-Lonergan et al., 1994b). Also, Anderson and Parrish (1989) noted a higher degree of titin degradation in tender steaks when compared with less tender steaks; however these results were questioned by Fritz et al. (1993) who suggested the myofibril preparation was causing some degree of titin degradation. An interesting band was noted in the 6 day postmortem aged control, NaCl/phosphate and  $\text{CaCl}_2$  steaks. That is a band appears directly below the T<sub>2</sub> band of titin. A third titin band has been seen and reported as T<sub>1-2</sub>, but this band is located between T<sub>1</sub> and T<sub>2</sub>, not below T<sub>2</sub>. It is also interesting that this band is no longer observed on gels by 13 days postmortem, leaving only T<sub>2</sub>. Moreover, this band located below T<sub>2</sub> does not appear on gels of myofibrils from NaCl/phosphate/ $\text{CaCl}_2$  injected steaks. The observed titin banding effect is very likely due to titin being digested by the calpain enzyme

system which would be activated by the addition of calcium to the meat system. This theory is in agreement with recent studies reported by Koohmaraie et al. (1988), and Wheeler et al. (1992) who observed increased tenderness by adding  $\text{CaCl}_2$  to ovine and bovine meat systems. It may be possible that the calcium in the  $\text{NaCl/phosphate/CaCl}_2$ -treated steaks was chelated by the phosphate, thus preventing degradation patterns of the other steaks, which prevented the occurrence of the same titin banding appearance.

It was observed that nebulin was completely degraded by three days postmortem in the  $\text{CaCl}_2$ -treated steaks as well as the  $\text{NaCl/phosphate/CaCl}_2$ -treated steaks, but is only partially degraded in the control and  $\text{NaCl/phosphate}$ -treated steaks. Huff-Lonergan et al. (1994b) noted that the rapid degradation of nebulin occurred as muscle was normally aged postmortem, but this is the first report of  $\text{CaCl}_2$ -treated steaks accelerating the degradation process of nebulin. There also appears to be a band at approximately 250,000 daltons, which is degraded from one band into two bands as the postmortem age is increased. This protein is in the approximate location of filamin dimers (Robson and Huiatt, 1983). It appears that this band may be affected by the  $\text{CaCl}_2$  treatment used in this study which implies that it is affected by the calpain enzyme system. This pattern is being further investigated in studies currently underway at Iowa State University.

Figure 2 is a 3-12% SDS-PAGE gel which shows treatment differences compared to a zero day control over all the aging times.

The  $\text{CaCl}_2$ -treated samples had the same banding patterns as previously discussed in Figure 1. The  $\text{NaCl}$ /phosphate/ $\text{CaCl}_2$ -treated samples had a slightly different titin degradation pattern, in that the band below  $T_2$  is not present, and the nebulin degradation pattern discussed is easily seen by comparing treatments.

A 12-20% SDS-PAGE gel is presented in Figure 3 which shows the  $\text{CaCl}_2$  treated samples have a darker 30,000 dalton component band by 3 days postmortem when compared with the control. This is indicative of proteolysis stimulated by calpains (Olson et al, 1977; Huff-Lonergan et al., 1994b). Figure 4 contains a 12-20% SDS-PAGE gel which shows the treatment differences existing for the 30,000 dalton component. From this gel, it is evident that the  $\text{CaCl}_2$ -treated samples have a more intense 30,000 dalton component by 3 and 6 days postmortem. These results suggest a stimulated activity of the calpain system by  $\text{Ca}^{++}$  which is in agreement with results of Cheng and Parrish (1977), Parrish et al. (1981), Koohmaraie (1992) and Huff-Lonergan et al. (1994b). The gradient gel system used in this study provided a large separation of closely spaced bands, which allows for easier identification of  $T_1$  and  $T_2$  as well as nebulin. It has been suggested that myofibril preparations, versus whole muscle preparations, promotes a higher degree of protein degradation (Fritz and Greaser, 1991); however, in our study as well as the study of Huff-Lonergan et al. (1994b) the myofibril preparation used did not cause any detectable degradation of proteins in comparison to whole muscle preparations

which were carried out on the same samples, collected at the same postmortem times.

Figure 5 shows the zero day control sample, indirect immunofluorescence results utilizing confocal laser microscopy. This microscope system uses laser technology to yield excellent resolution at higher magnifications and also create three-dimensional images, while eliminating out of focus images. Images created with these microscopes can be superimposed onto each other, which is very useful when dual staining is applied to samples (Huff-Lonergan et al., 1994a). Also, this microscope is able to scan through cross-sections of myofibrils, which allows researchers to confirm that antibody staining is throughout the entire myofibril and not just on the outer surfaces. Results indicate that initially there are two stained titin bands per sarcomere located in the A-I junction area. The paired bands represent the labeling of a single epitope on the titin molecule and they are divided by the Z-line label which is immuno-stained bovine desmin. Several researchers have also observed the same type of banding pattern (Wang and Greaser, 1985; Ringkob et al., 1988; Beekman and Ringkob, 1992). Figure 6 illustrates that by 3 days postmortem, the staining is very near the Z-line in the control, and all the treated samples (A, B, C and D). It is extremely difficult to detect any treatment differences, most probably due to a cleavage of the titin molecule near the A-band edge, therefore allowing the elastic portion of titin to collapse on the Z-line shortly after the zero day sample was collected. The pattern observed in the 3 day postmortem aged steaks

was identical in both the 6 and 13 day aged steaks; however, the myofibrils fragmented considerably over the postmortem aging periods, ranging from 15 to 20 sarcomeres at 3 days postmortem to approximately 5 sarcomeres by 6 and 13 days postmortem. This observation is in agreement with previous indirect immunofluorescence studies (Olson et al., 1976; Ringkob et al. 1988; Beekman and Ringkob, 1992).

The MFI, WHC and cook loss means are listed in Tables 4, 5 and 6. The higher MFI values indicate an increase in proteolysis and tenderness when  $\text{CaCl}_2$  was injected and aged 3 and 6 days postmortem ( $P < .05$ ). By 13 days postmortem, the MFI values had begun to level out among the treatments with the  $\text{CaCl}_2$  and control groups having the highest MFI values ( $P < .05$ ). Olson et al. (1976) and Olson et al. (1977) also observed an increase in the MFI as tenderization occurred postmortem. Both of the treatments containing phosphate exhibited a decrease in MFI values compared to the  $\text{CaCl}_2$  and control, perhaps due to a chelation of calcium by the injected phosphate. The WHC data shows a significant difference ( $P < .05$ ) between the NaCl/phosphate/ $\text{CaCl}_2$  steak, the other treated steaks and the control at three days postmortem (Table 4). Table 5 shows that by 6 days postmortem both the NaCl/phosphate and NaCl/phosphate/ $\text{CaCl}_2$  had significantly lower WHC values (increased water holding ability) than the  $\text{CaCl}_2$  or control samples, and at 13 days postmortem all the treated groups had significantly lower ( $P < .05$ ) WHC values than the control, with the NaCl/phosphate and NaCl/phosphate/ $\text{CaCl}_2$  having the

lowest values. These results indicate that the NaCl containing treatments held more water which resulted in the lower WHC values. This increase in WHC is probably due to a swelling of the myofibrils in the presence of NaCl and phosphate which can occur because the structural constraints are removed (Offer and Trinick, 1983; Paterson et al., 1988). Specifically, NaCl enhances the WHC since the Cl<sup>-</sup> anions are attracted to the amino groups of the muscle proteins. This causes the protein to have a higher negative charge and an increase in repulsion. The increased repulsion allows more water binding sites to be exposed (Whiting, 1988). NaCl will also shift the isoelectric point down which increases the negative charge of carboxyl groups resulting in more space for water binding (Acton et al., 1983). The CaCl<sub>2</sub>-treated samples had lower WHC possibly because divalent cations form cross bridges between peptide chains, and they will also bond with carboxyl, sulfhydryl and hydroxyl groups on proteins (Asghar et al., 1985). The bonds which are formed can prevent the presentation of water binding sites on the proteins, and therefore may act to hold the myofibril together.

Table 1. Least squares means and standard errors of various sensory attributes of beef longissimus steaks injected with one of four treatments stored three days post-mortem.<sup>1</sup>

Attribute	Water Control	NaCl/phosphate	CaCl <sub>2</sub>	NaCl/CaCl <sub>2</sub> / Phosphate	Std Err
Softness to tooth pressure	6.35 <sup>a</sup>	10.57 <sup>b</sup>	7.76 <sup>a</sup>	10.09 <sup>b</sup>	1.14
Fiber Fragmentation	5.08 <sup>a</sup>	9.91 <sup>b</sup>	6.61 <sup>a</sup>	9.51 <sup>b</sup>	1.32
Juiciness	8.99 <sup>a</sup>	11.94 <sup>b</sup>	9.43 <sup>a</sup>	10.98 <sup>b</sup>	0.48
Bitterness	2.52 <sup>a</sup>	2.96 <sup>a</sup>	4.24 <sup>b</sup>	2.54 <sup>a</sup>	0.37
Saltiness	1.61 <sup>a</sup>	7.76 <sup>b</sup>	1.60 <sup>a</sup>	8.18 <sup>b</sup>	0.48

<sup>ab</sup> Means within rows with different superscripts are significantly different ( $P < .05$ ).

<sup>1</sup> Units reported represent means from values collected on a 15 cm scale (0=minimum, 15=maximum).



Table 2. Least squares means and standard errors of various sensory attributes of beef longissimus steaks injected with one of four treatments stored six days post-mortem.<sup>1</sup>

Attribute	Water Control	NaCl/phosphate	CaCl <sub>2</sub>	NaCl/CaCl <sub>2</sub> /Phosphate	Std Err
Softness to tooth pressure	6.33 <sup>a</sup>	10.01 <sup>b</sup>	8.73 <sup>b</sup>	10.36	1.14
Fiber Fragmentation	4.92 <sup>a</sup>	9.44 <sup>b</sup>	8.74 <sup>b</sup>	10.03 <sup>b</sup>	1.32
Juiciness	9.25 <sup>a</sup>	10.77 <sup>b</sup>	9.97 <sup>ab</sup>	10.36 <sup>ab</sup>	0.48
Bitterness	2.20 <sup>a</sup>	3.08 <sup>ab</sup>	3.87 <sup>b</sup>	2.81 <sup>ab</sup>	0.37
Saltiness	1.58 <sup>a</sup>	5.66 <sup>b</sup>	2.98 <sup>a</sup>	8.13 <sup>c</sup>	0.48

<sup>abc</sup> Means within rows with different superscripts are significantly different ( $P < .05$ ).

<sup>1</sup> Units reported represent means from values collected on a 15 cm scale (0=minimum, 15=maximum).

Table 3. Least squares means and standard errors of various sensory attributes of beef longissimus steaks injected with one of four treatments stored thirteen days post-mortem.<sup>1</sup>

Attribute	Water Control	NaCl/phosphate	CaCl <sub>2</sub>	NaCl/CaCl <sub>2</sub> / Phosphate	Std Err	100
Softness to tooth pressure	9.76 <sup>a</sup>	10.78 <sup>ab</sup>	10.46 <sup>a</sup>	12.65 <sup>b</sup>	1.14	
Fiber Fragmentation	9.03 <sup>a</sup>	10.98 <sup>a</sup>	10.02 <sup>a</sup>	12.57 <sup>b</sup>	1.32	
Juiciness	10.40 <sup>a</sup>	10.97 <sup>ab</sup>	10.73 <sup>ab</sup>	12.20 <sup>b</sup>	0.48	
Bitterness	2.38 <sup>a</sup>	3.44 <sup>ab</sup>	4.30 <sup>b</sup>	2.91 <sup>a</sup>	0.37	
Saltiness	1.70 <sup>a</sup>	6.50 <sup>b</sup>	2.76 <sup>a</sup>	7.89 <sup>c</sup>	0.48	

<sup>abc</sup> Means within rows with different superscripts are significantly different ( $P < .05$ ).

<sup>1</sup> Units reported represent means from values collected on a 15 cm scale (0=minimum, 15=maximum).

Table 4. Least squares means and standard errors of various attributes of beef longissimus steaks injected with one of four treatments and stored three days post-mortem.

Attribute	Water Control	NaCl/phosphate	CaCl <sub>2</sub>	NaCl/CaCl <sub>2</sub> / Phosphate	Std Err
Warner-Bratzler shear (Kg)	6.68 <sup>a</sup>	4.77 <sup>b</sup>	4.99 <sup>b</sup>	4.36 <sup>b</sup>	0.15
Myofibril fragmentation index	53.47 <sup>ab</sup>	45.07 <sup>c</sup>	59.67 <sup>a</sup>	49.93 <sup>b</sup>	12.48
Water holding capacity (water:meat)	2.22 <sup>a</sup>	2.13 <sup>a</sup>	2.18 <sup>a</sup>	2.02 <sup>b</sup>	0.01
Cook yield (% yield)	64.47 <sup>a</sup>	67.23 <sup>a</sup>	62.89 <sup>a</sup>	66.83 <sup>a</sup>	5.74

<sup>ab</sup> Means within rows with different superscripts are significantly different ( $P < .05$ ).

Table 5. Least squares means and standard errors of various attributes of beef longissimus steaks injected with one of four treatments stored six days post-mortem.

Attribute	Water Control	NaCl/phosphate	CaCl <sub>2</sub>	NaCl/CaCl <sub>2</sub> / Phosphate	Std Err
Warner-Bratzler shear (Kg)	5.88 <sup>a</sup>	3.74 <sup>b</sup>	4.19 <sup>b</sup>	3.34 <sup>b</sup>	0.15
Myofibril fragmentation index	54.46 <sup>ab</sup>	51.39 <sup>a</sup>	60.07 <sup>b</sup>	55.93 <sup>ab</sup>	12.48
Water holding capacity (water:meat)	2.22 <sup>a</sup>	1.99 <sup>b</sup>	2.05 <sup>ab</sup>	2.00 <sup>b</sup>	0.01
Cook yield (% yield)	66.82 <sup>a</sup>	70.9 <sup>a</sup>	69.02 <sup>a</sup>	70.50 <sup>a</sup>	5.74

<sup>ab</sup> Means within rows with different superscripts are significantly different ( $P < .05$ ).

Table 6. Least squares means and standard errors of various attributes of beef longissimus steaks injected with one of four treatments stored thirteen days post-mortem.

Attribute	Water Control	NaCl/phosphate	CaCl <sub>2</sub>	NaCl/CaCl <sub>2</sub> / Phosphate	Std Err
Warner-Bratzler shear (Kg)	5.21 <sup>a</sup>	2.76 <sup>b</sup>	3.42 <sup>b</sup>	2.49 <sup>b</sup>	0.15
Myofibril fragmentation index	64.40 <sup>a</sup>	54.6 <sup>b</sup>	62.66 <sup>a</sup>	56.67 <sup>b</sup>	12.48
Water holding capacity (water:meat)	2.35 <sup>a</sup>	1.81 <sup>b</sup>	2.09 <sup>c</sup>	1.71 <sup>b</sup>	0.01
Cook yield (% yield)	65.35 <sup>a</sup>	69.25 <sup>a</sup>	68.34 <sup>a</sup>	68.86 <sup>a</sup>	5.74

<sup>ab</sup> Means within rows with different superscripts are significantly different ( $P < .05$ ).

**Figure 1.** 3-12% SDS-gradient gel (Coomassie blue stained) of purified myofibrils from beef longissimus steaks injected with water control and  $\text{CaCl}_2$  over all the post-mortem aging times. STD= molecular weight standards; 0, 3, 6, 13= days aged postmortem; T= purified bovine titin; N= purified bovine nebulin.

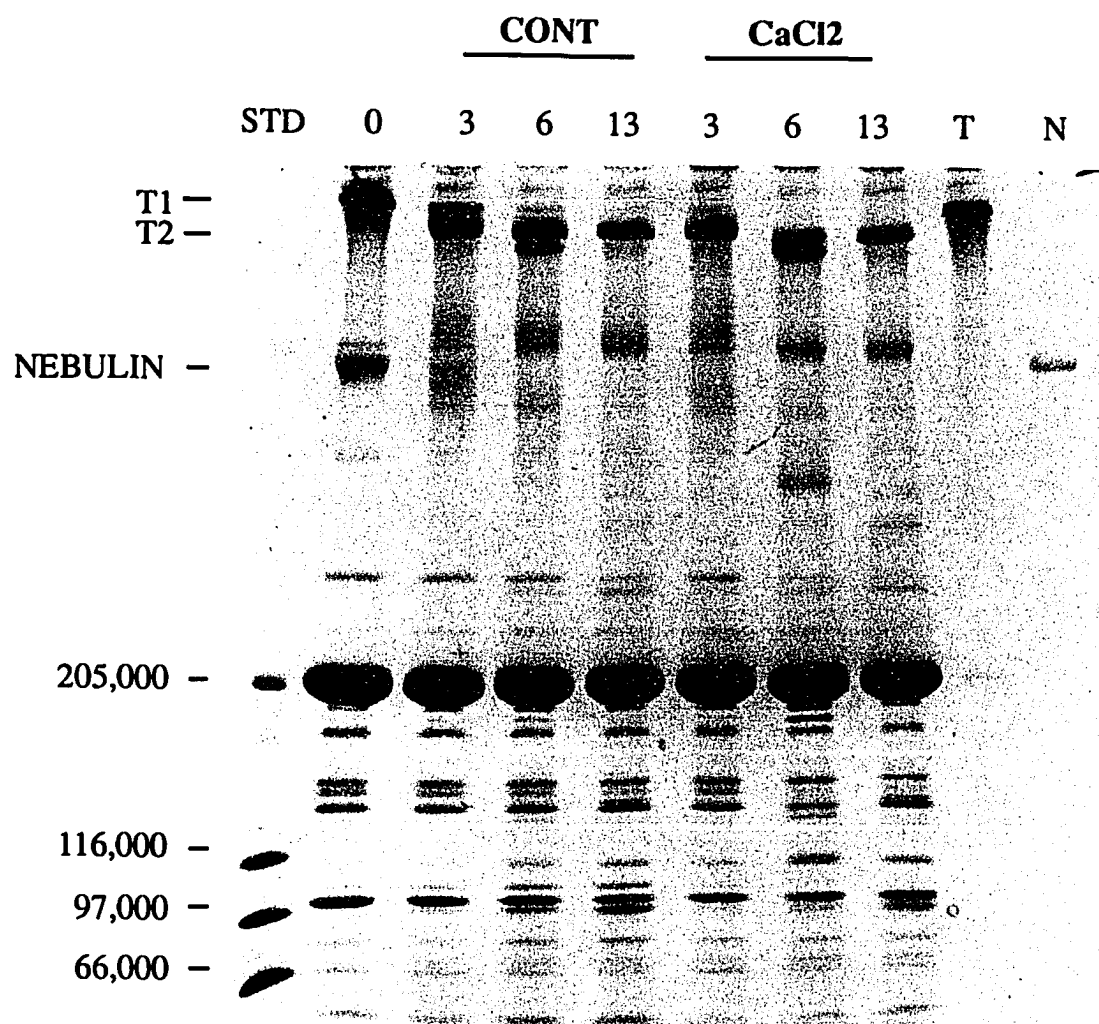
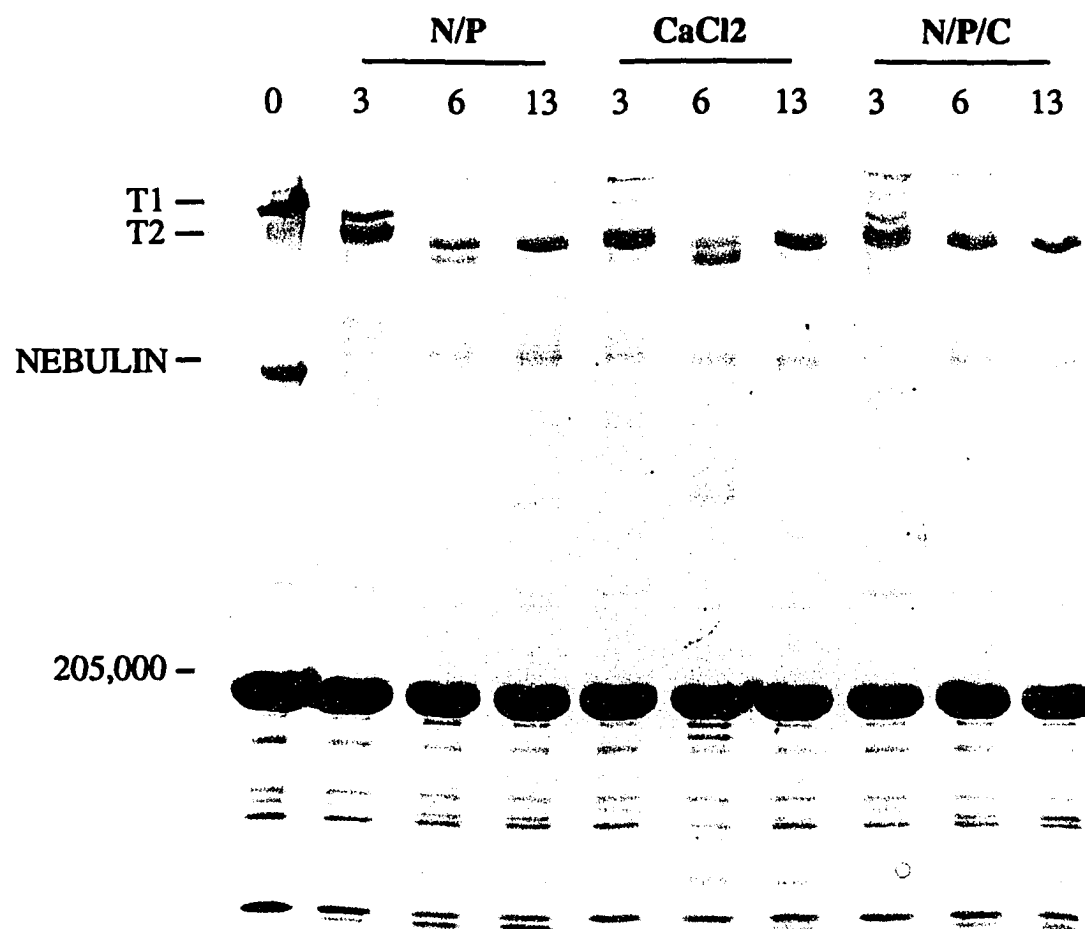


Figure 2. 3-12% SDS-gradient gel (Coomassie blue stained) of purified myofibrils from beef longissimus steaks injected with NaCl/phosphate (N/P),  $\text{CaCl}_2$ , and NaCl/phosphate/ $\text{CaCl}_2$  (N/P/C) over all the post-mortem aging times. STD= molecular weight standards; 0 3,6,13= days aged postmortem; T= purified bovine titin; N= purified bovine nebulin.





**Figure 3.** 12-20% SDS-gradient gel (Coomassie blue stained) of purified myofibrils from beef longissimus steaks injected with water control and  $\text{CaCl}_2$  over all the post-mortem aging times. STD= molecular weight standards; 0, 3, 6, 13= days aged postmortem; T= purified bovine titin; N= purified bovine nebulin.

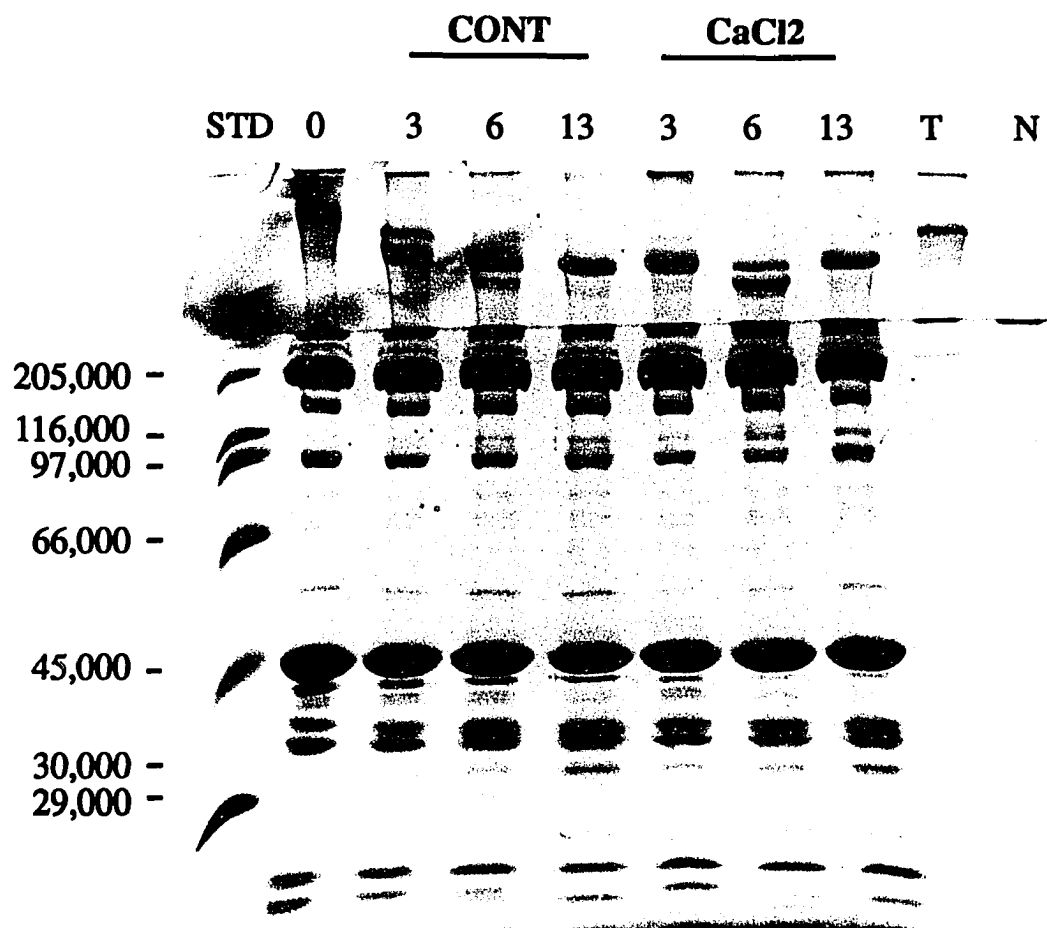


Figure 4. 12-20% SDS-gradient gel (Coomassie blue stained) of purified myofibrils from beef longissimus steaks injected with NaCl/phosphate (N/P),  $\text{CaCl}_2$ , and NaCl/phosphate/ $\text{CaCl}_2$  (N/P/C) over all the post-mortem aging times. STD= molecular weight standards; 0, 3, 6, 13= days aged postmortem; T= purified bovine titin; N= purified bovine nebulin.

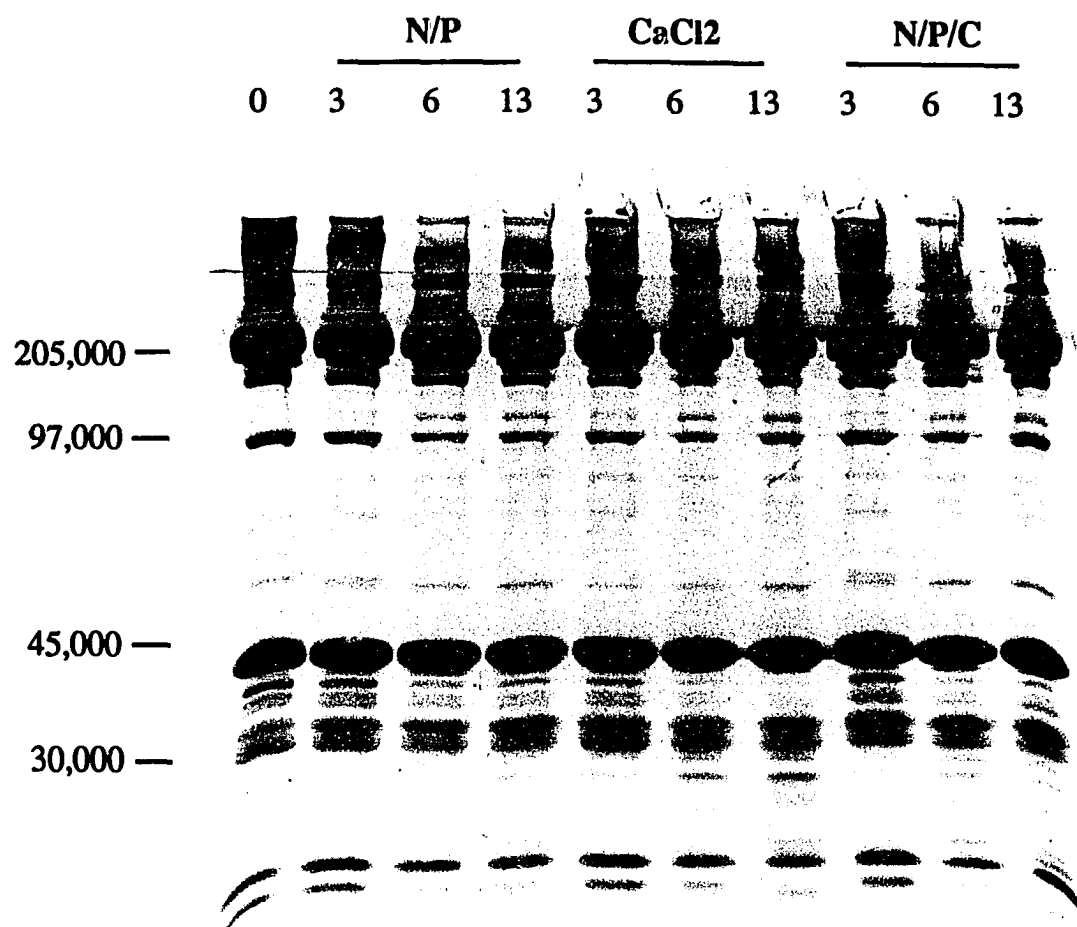


Figure 5. Indirect immunofluorescent image of purified myofibrils from beef longissimus steaks aged zero days (30 minutes) postmortem dual labeled with anti-desmin (Z-line label), and anti-titin (4C7). Z=Z-line. The titin staining is represented by green, and the desmin staining by red staining. A yellow color indicates the stained areas are in alignment.

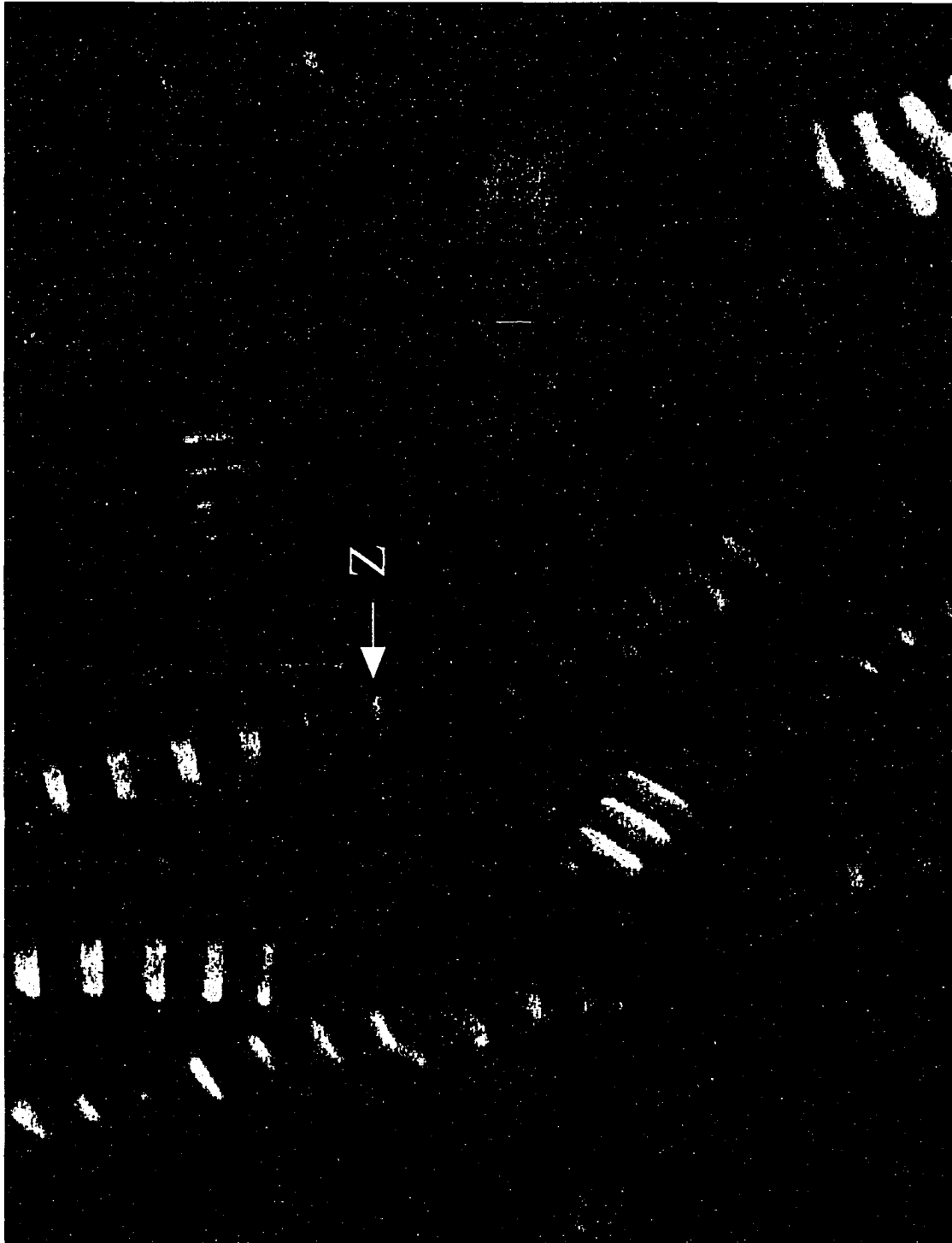
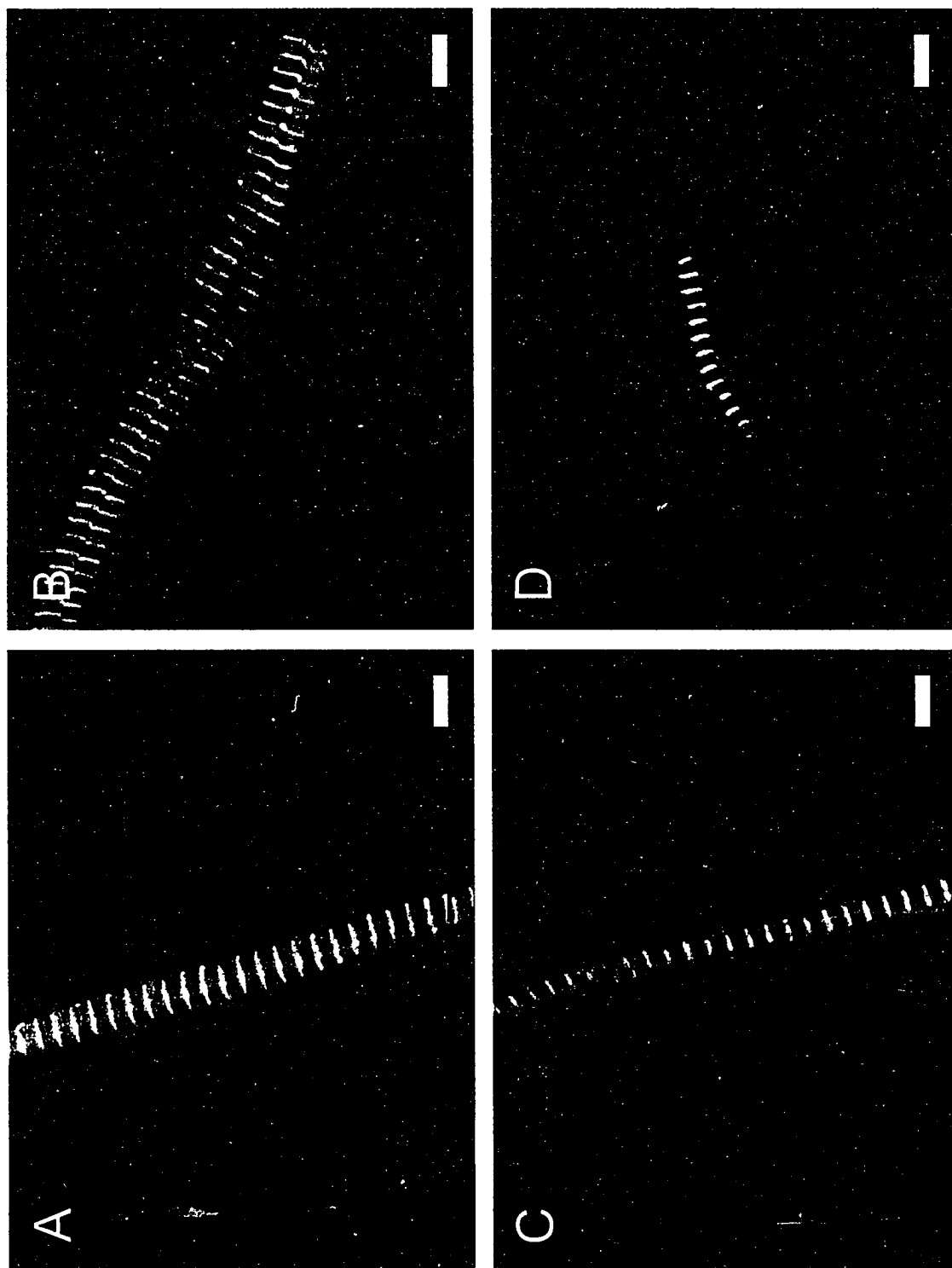


Figure 6. Indirect immunofluorescent image of purified myofibrils from beef longissimus steaks aged three days postmortem, and injected with: A) water control, B) NaCl/phosphate, C)  $\text{CaCl}_2$ , and D) NaCl/phosphate/ $\text{CaCl}_2$  and dual labeled with anti-desmin (Z-line label), and anti-titin (4C7). The titin staining is represented by green, and the desmin staining by red staining. A yellow color indicates the stained areas are in alignment. The white bar is 5 microns in length.





## CONCLUSIONS

Based on this study of the effects of water (control), NaCl/phosphate, and  $\text{CaCl}_2$  treatments on beef loin steaks injected to a 10% level at 24 hours postmortem and stored 3, 6 or 13 days postmortem, the following conclusions can be made: 1) Steaks injected with NaCl/phosphate were the most tender at 3 days postmortem, 2) Steaks injected with  $\text{CaCl}_2$  were more tender than controls, but had the highest bitterness scores, 3)  $\text{CaCl}_2$ -treated steaks were tenderized by proteolysis, 4) NaCl/phosphate-treated steaks were tenderized by enhanced WHC, 5) Tenderness was similar for the treated steaks at 6 and 13 days postmortem, but were significantly more tender than the control, 6) NaCl/phosphate would seem to hold a greater potential for commercial application, and 7) More research needs to be done on the flavor of  $\text{CaCl}_2$  injected steaks by a consumer panel to determine palatability acceptance of the bitterness component.

## REFERENCES

- Anderson, T.J. and Parrish, F.C., Jr. 1989. Postmortem degradation of titin and nebulin of beef steaks varying in tenderness. *J. Food Sci.* 54:748.
- Acton, J.C., Ziegler, G.R. and Burge, D.L. 1983. Functionality of muscle constituents in the processing of comminuted meat products. *Crit. Rev. Food Sci. Nutr.* 18:99.
- Asghar, A., Samejima, K. and Yasui, T. 1985. Functionality of muscle proteins in gelation mechanisms of structured meat products. *Crit. Rev. Food Sci. Nutr.* 22:27.
- Bartoshuk, L.M. 1979. Bitter taste of saccharin related to the genetic ability to taste the bitter substance 6-n-propylthiouracil. *Science* 205:934.
- Beekman, D.D. and Ringkob, T.P. 1992. Confocal fluorescence microscopy of immunolabeled titin and alpha-actinin in beef myofibrils. *J. Anim. Sci.* 70(suppl):114.
- Benito-Delgado, J., Marriott, N.G., Claus, J.R., Wang, H., and Graham, P.P. 1994. Chuck longissimus and infraspinatus muscle characteristics as affected by rigor state, blade tenderization and calcium chloride injection. *J. Food Sci.*, 59:295.
- Booren, A.M., Jones, K.W., Mandigo, R.W. and Olson, D.G. 1981. Effects of blade tenderization, vacuum mixing, salt addition and mixing time on binding of meat pieces into sectioned and formed beef steaks. *J. Food Sci.* 46:1678.
- Bouton, P.E., Ford, A.L., Harris, P.V. and Shaw, F.E. 1980. Electrical stimulation of beef sides. *Meat Sci.* 4:145.
- Cheng, C.S. and Parrish, F.C., Jr. 1977. Effects of  $\text{Ca}^{2+}$  on changes in myofibrillar proteins of bovine skeletal muscle. *J. Food Sci.* 42:1621.
- Davey, C.L. and Graahuis, A.E. 1976. Structural changes in beef muscle during aging. *J. Sci. Food Agric.* 26:301.

- de Pulgar, M.C. 1982. Relationships of beef carcass grade factors to certain physical and sensory characteristics of bovine longissimus muscle. Master Thesis, Iowa State University.
- Dransfield, E. 1993. Modelling postmortem tenderization - IV: Role of calpains and calpastatin in conditioning. *Meat Sci.* 34:217.
- Dutson, T.R. 1983. Relationship of pH and temperature to disruption specific muscle proteins and activity of lysosomal proteases. *J. Food Biochem.* 7:223.
- Fritz, J.D. and Greaser, M.L. 1991. Changes in titin and nebulin in postmortem bovine muscle revealed by gel electrophoresis, Western blotting and immunofluorescence microscopy. *J Food Sci.* 56:607.
- Fritz, J.D., Mitchell, M.C., Marsh, B.B. and Greaser, M.L. 1993. Titin content of beef in relation to tenderness. *Meat Sci.* 33:41.
- Glanville, E.V. and Kaplan, A.R. 1965. Food preferences and sensitivity of taste for bitter compounds. *Nature* 205:851.
- Goll, D.E., Thompson, V.F., Taylor, R.G. and Zalewska, T. 1992. Is calpain activity regulated by membranes and autolysis or by calcium and calpastatin? *Bio Essays* 14:549.
- Grau, R. and Hamm, R. 1953. A simple method for the detection of water-binding in muscle. *Naturwissenschaften.* 40:29.
- Harris, H., and Kalmus, H. 1949. The measurement of taste sensitivity to phenylthiourea (P.T.C.). *Annu. Eugenics Lond.* 15:24.
- Honikel, K.O., Kim, C.J., Hamm, R. and Roncales, P. 1986. Sarcomere shortening of pre-rigor muscles and its influence on drip loss. *Meat Sci.* 16:267.
- Huff, E.J., and F.C. Parrish, Jr. 1993. Bovine longissimus muscle tenderness as affected by postmortem aging time, animal age and sex. *J. Food Sci.* 58:713.

- Huff-Lonergan, E.J., Beekman, D.D. and Parrish, F.C., Jr. 1994a. Protein separation and analysis of certain skeletal muscle proteins: Principles and techniques. In Protein Functionality in Food Systems, (Ed) Hettiarachchy, N.S., Zeigler, G.R. p. 79-119. Marcel Dekker Inc., New York.
- Huff-Lonergan, E.J., Parrish, F.C., Jr. and Robson, R.M. 1994b. Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. J. Anim. Sci. (submitted).
- Koohmaraie, M. 1992. Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle m-calpain. J. Anim. Sci. 70:3071.
- Koohmaraie, M., Babiker, A.S., Merkel, R.A. and Dutson, T.R. 1988a. The role of  $\text{Ca}^{2+}$  dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. 53:1253.
- MacBride, M.A. and Parrish, F.C., Jr. 1977. The 30,000 Dalton component of tender bovine longissimus muscle. J. Food Sci. 42:1627.
- Marsh, B.B. 1983. Effects of early postmortem muscle pH and temperature on meat tenderness. Proc. Recip. Meat Conf. 36:131.
- Marsh, B.B., Ringkob, T.P., Russell, R.L., Swartz, D.R. and Pajel, L.A. 1987. Effects of early postmortem glycolytic rate on beef tenderness. Meat Sci. 21:241.
- McKeith, F.K., Savell, J.W. and Smith, G.C. 1981. Tenderness improvement of the major muscles of the beef carcasses by electrical stimulation. J. Food Sci. 46:1774.
- Morgan, J.B., Miller, R.K., Mendez, F.M., Hale, D.S. and Savell, J.W. 1991. Using calcium chloride injection to improve tenderness of beef from mature cows. J. Anim. Sci. 69:4469.
- Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Sci. 8:245.

- Olson, D.G., Parrish, F.C., Jr., Dayton, W.R. and Goll, D.E. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. *J. Food Sci.* 42:117.
- Olson, D.G., Parrish, F.C., Jr. and Stromer, M.H. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* 41:1036.
- Parrish, F.C., Jr., Young, R.B., Miner, B.E. and Anderson, L.D. 1973. Effect of postmortem conditions on certain chemical, morphological and organoleptic properties of bovine muscle. *J. Food Sci.* 38:690.
- Parrish, F.C., Jr., Selvig, C.J., Culler, R.D. and Zeece, M.G. 1981. CAF activity, calcium concentration, and the 30,000-dalton component of tough and tender bovine longissimus muscle. *J. Food Sci.* 46:308.
- Paterson, B.C., Parrish, F.C., Jr. and Stromer, M.H. 1988. Effects of salt and pyrophosphate on the physical and chemical properties of beef muscles. *J. Food Sci.* 53:1258.
- Pike, M.M., Ringkob, T.P., Beekman, D.D., Koh, Y.O. and Gerthoffer, W.T. 1993. Quadratic relationship between early postmortem glycolytic rate and beef tenderness. *Meat Sci.* 34:13.
- Ringkob, T.P., Marsh, B.B. and Greaser, M.L. 1988. Change in titin position in postmortem bovine muscle. *J. Food Sci.* 53:276.
- Robson, R.M., Goll, D.E. and Temple M.J. 1968. Determination of protein in "tris" buffer by the biuret reaction. *Anal. Biochem.* 24:339.
- Robson, R.M. and Huiatt, T.W. 1983. Roles of the cytoskeletal proteins desmin, titin and nebulin in muscle. p. 116 in *Proceedings of the 36th Reciprocal Meat Conference*. Fargo, ND. National Livestock and Meat Board, Chicago, IL.
- Rolan, T.L., Davis, G.W., Seideman, S.C., Wheeler, T.L. and Miller, M.F. 1988. Effects of blade tenderization and proteolytic enzyme on restructured steaks from beef bullock chucks. *J. Food Sci.* 53:1062.
- SAS. 1988. *SAS/STAT User's guide*. Release 6.03. SAS Inst., Cary, NC.

- Savell, J.W., Smith, G.C., Carpenter, Z.L. and Parrish, F.C., Jr. 1979. Influence of electrical stimulation on certain characteristics of heavy weight beef carcasses. *J. Food Sci.* 44:911.
- Shults, G.W., Russell, D.R. and Wierbicki, E. 1972. Effect of condensed phosphates on pH, swelling and water-holding capacity of beef. *J. Food Sci.* 37:860.
- St. Angelo, A.J., Koohmaraie, M., Crippen, K.L. and Crouse, J. 1991. Acceleration of tenderization/inhibition of warmed over flavor by calcium chloride antioxidant infusion into lamb carcasses. *J. Food Sci.* 56:359.
- Tatsumi, R. and Takahashi, K. 1992. Calcium induced fragmentation of skeletal muscle nebulin filaments. *J. Biochem.* 112:775.
- Trout, G.R. and Schmidt, G.R. 1983. Utilization of phosphates in meat products. *Proc. Recip. Meat Conf.* 36:24.
- Wang, K. 1982. Purification of titin and nebulin. *Methods Enzymol.* 85:17.
- Wang, K. and Greaser, M.L. 1985. Immunocytochemical studies using a monoclonal antibody to bovine cardiac titin on intact and extracted myofibrils. *J. Muscle Res. Cell Motil.* 6:293.
- Wang, K., McClure, J., and Tu, A. 1979. Titin a major myofibrillar component of striated muscle. *Proc. Natl. Acad. Sci. USA.* 76:3698.
- Wheeler, T.L., Koohmaraie, M. and Crouse, J.D. 1991. Effects of calcium chloride injection and hot boning on the tenderness of round muscles. *J. Anim. Sci.* 69:4871.
- Wheeler, T.L., Koohmaraie, M., Lansdell, J.L., Siragusa, G.R. and Miller, M.F. 1993. Effects of postmortem injection time, injection level, and concentration of calcium chloride on beef quality traits. *J. Anim. Sci.* 71:2965.

- Whipple, G. and Koohmaraie, M. 1993. Calcium chloride marination effects on beef steak tenderness and calpain proteolytic activity. *Meat Sci.* 33:265.
- Whiting, R.C. 1988. Ingredients and processing factors that control muscle protein functionality. *Food Technol.* 42:104.
- Zeece, M.G., Robson, R.M., Lusby, M.L. and Parrish, F.C., Jr. 1986. Effect of calcium activated protease (CAP) on bovine myofibrils under differed conditions of pH and temperature. *J. Food Sci.* 51:707.



### GENERAL SUMMARY

There is a need for tenderness uniformity in the fresh beef products sold today. If a method of introducing solutions containing ions to enhance tenderness and, which does not impart negative flavor attributes could be identified it would be useful to a large segment of the meat industry. Loin steaks were injected with water (control) and solutions of sodium chloride (NaCl), tripolyphosphate and calcium chloride ( $\text{CaCl}_2$ ) at one day postmortem, and aged 3, 6 and 13 days postmortem at 2°C. The data showed a significant improvement in tenderness for all the treated groups compared with the control as was indicated by lower WB shear values and higher sensory scores for softness to tooth pressure and fiber fragmentation. The NaCl/phosphate and NaCl/phosphate/ $\text{CaCl}_2$ -treated groups exhibited the lowest WB shears and had an improvement of approximately 30% over the control WB shear values at 3 days postmortem. Although the  $\text{CaCl}_2$  treatment improved tenderness, the sensory panel detected increased bitterness in the  $\text{CaCl}_2$ -treated steaks at each postmortem aging period, which makes the widespread use of  $\text{CaCl}_2$  injection treatments unlikely.  $\text{CaCl}_2$ -treated steaks had higher MFI values at 3 and 6 days postmortem compared with the control steaks. SDS-PAGE analysis illustrated that  $\text{CaCl}_2$  increased the rate of degradation of titin, and the rate of appearance of the 30,000-dalton component in contrast to other samples. Indirect immunofluorescence showed as the postmortem age increased from zero days to 3 days there was a degradation of the titin molecule from two bands per sarcomere to one

band per sarcomere located adjacent to the Z-line in all the treatments. The NaCl/phosphate and NaCl/phosphate/CaCl<sub>2</sub> had consistently higher WHC compared with the control and CaCl<sub>2</sub>-treated steaks over all the postmortem aging times. At 6 and 13 days postmortem all treatments had similar palatability values, but were significantly different in palatability from the water injected control. The results of this study suggest the mechanism of the tenderization process for CaCl<sub>2</sub>-treated steaks was enhanced proteolysis, whereas the mechanism of the tenderization process for NaCl/phosphate was increased WHC. Both the NaCl/phosphate and CaCl<sub>2</sub> treatments improved tenderness; however, because 20% of the population can detect the bitterness of CaCl<sub>2</sub>, the NaCl/phosphate treatment would have a greater possibility for commercial applications because of more familiar, and favorable attributes.

## GENERAL REFERENCES

- Anderson, T.J. and Parrish, F.C., Jr. 1989. Postmortem degradation of titin and nebulin of beef steaks varying in tenderness. *J. Food Sci.* 54:748.
- Arnold, N., Wierbicki, E. and Deatherage, F.E. 1956. Postmortem changes in the interactions of cations and proteins of beef and their relations to sex and diethylstilbestrol treatment. *Food Tech.* 10:245.
- Asghar, A., Samejima, K. and Yasui, T. 1985. Functionality of muscle proteins in gelation mechanisms of structured meat products. *Crit. Rev. Food Sci. Nutr.* 22:27.
- Bailey, A.J. 1972. The basis of meat texture. *J. Sci. Food Agric.* 23:995.
- Bailey, A.J. 1989. The chemistry of collagen cross-links and their role in meat texture. *Proc. Recip. Meat Conf.* 42:127.
- Bailey, K. 1948. Tropomyosin: A new asymmetric protein component of the muscle fibril. *Biochem. J.* 43:241.
- Bechtel, P.J. and Parrish, F.C., Jr. 1983. Effect of postmortem storage and temperature on muscle protein degradation: Analysis by SDS-gel electrophoresis. *J. Food Sci.* 48:294.
- Beekman, D.D. and Parrish, F.C., Jr. 1993. A comparative study of water holding capacity techniques. *Proc. Recip. Meat Conf.* 45:203.
- Beekman, D.D. and Parrish, F.C., Jr. 1994. Comparison of meat aging systems: European box versus vacuum packaging. 1994 Beef Research Report. Iowa State University. March, 1994. p. 136.
- Beekman, D.D. and Ringkob, T.P. 1992. Confocal fluorescence microscopy of immunolabeled titin and alpha-actinin in beef myofibrils. *J. Anim. Sci.* 70(suppl):114.
- Bendall, J.R. 1967. The elastin content of various muscles of beef animals *J. Sci. Food Agric.* 18:553.

- Bendall, J.R. 1978. Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses. *Meat Sci.* 2:91.
- Benito-Delgado, J., Marriott, N.G., Claus, J.R., Wang, H. and Graham, P.P. 1994. Chuck longissimus and infraspinatus muscle characteristics as effected by rigor state, blade tenderization and calcium chloride injection. *J. Food Sci.* 59:295.
- Bennett, P., Craig, R., Starr, R., and Offer, G. 1986. The ultrastructural location of C-protein, X-protein and H-protein in rabbit muscle. *J. Musc. Res. Cell Motil.* 7:550.
- Berry, B.W. 1986. Development of a texture profile panel for evaluating restructured beef steaks varying in meat particle size. *J. Sensory Stud.* 1:15.
- Bilgili, S.F., Egbert, W.R. and Huffman, D.L. 1989. Effect of postmortem aging temperature on sarcomere length and tenderness of broiler pectoralis major. *Poultry Sci.* 68:1588.
- Booren, A.M., Jones, K.W., Mandigo, R.W. and Olson, D.G. 1981. Effects of blade tenderization, vacuum mixing, salt addition and mixing time on binding of meat pieces into sectioned and formed beef steaks. *J. Food Sci.* 46:1678.
- Bouton, P.E., Ford, A.L., Harris, P.V. and Shaw, F.E. 1980. Electrical stimulation of beef sides. *Meat Sci.* 4:145.
- Bouton, P.E., Harris, P.V. and Shorthose, W.R. 1971. Effect of ultimate pH upon the water holding capacity and tenderness of mutton. *J. Food Sci.* 36:435.
- Bouton, P.E., Harris, P.V. and Shorthose, W.R. 1972. The effects of ultimate pH on ovine muscle: water holding capacity. *J. Food Sci.* 37:351.
- Bratzler, L.J. 1932. Measuring the tenderness of meat by means of a mechanical shear. Kansas State College, Master of Science Thesis. Manhattan, KS.

- Brown, L.M. and Zayas, J.F. 1990. Corn germ protein flavor as an extender in broiled beef patties. *J. Food Sci.* 55:888.
- Busch, W.A., Parrish, F.C., Jr. and Goll, D.E. 1967. Molecular properties of postmortem muscle. 4. Effect of temperature on adenosine triphosphate degradation, isometric tension parameters, and shear resistance of bovine muscle. *J. Food Sci.* 32:1.
- Busch, W.A., Stromer, M.H., Goll, D.E. and Suzuki, A. 1972.  $\text{Ca}^{2+}$  specific removal of Z-lines from rabbit skeletal muscle. *J. Cell Biol.* 52:367.
- Calkins, C.R. and Seideman. 1988. Relationships among calcium-dependent protease, cathepsins B and H, meat tenderness and the response of muscle to aging. *J. Anim. Sci.* 66:1186.
- Cheng, C.S. and Parrish, F.C., Jr. 1977. Effects of  $\text{Ca}^{2+}$  on changes in myofibrillar proteins of bovine skeletal muscle. *J. Food Sci.* 42:1621.
- Chrystall, B.B. and Devine, C.E. 1982. Electrical stimulation in New Zealand. *Procedure Meat Science Technology Symposium* (Franklin, K.R., Cross, H.R., eds.) Nat. Livestock and Meat Board. p. 115. Chicago, IL.
- Cross, H.E., Smith, G.C., Kotula, A.W., and Muse, D.A. 1979. Effects of electrical stimulation and shrouding method on quality and palatability of beef carcasses. *J. Food Sci.* 44:1560.
- Cross, H.R., Bertholdt, M.F., Dikeman, M.E., Greene, B.P., Moody, W.G., Stagge, R. and West, R.L. 1978. Recommended guidelines for cookery and sensory evaluation of meat. Ad-hoc committee on guidelines for cookery and sensory evaluation of meat, American Meat Science Association (AMSA), Auburn, AL. and National Livestock and Meat Board, Chicago, IL.
- Cross, H.R., Carpenter, Z.L. and Smith, G.C. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. *J. Food Sci.* 38:998.

- Culler, R.D., Parrish, F.C., Jr., Smith, G.C. and Cross, H.R. 1978. Relationship of myofibrillar fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. *J. Food Sci.* 43:1177.
- Dagbjartsson, B. and Solberg, M. 1972. A simple method to determine the water-holding capacity of muscle foods. *J. Food Sci.* 37:499.
- Davey, C.L. 1983. Post mortem chemical changes in muscle meat aging. p. 108 in *Proceedings of the 36th Reciprocal Meat Conference*. Fargo, ND. National Livestock and Meat Board, Chicago, IL.
- Davey, C.L. and Gilbert, K.V. 1969. Studies in meat tenderness. 7. Changes in the fine structure of meat during aging. *J. Food Sci.* 34:69.
- Davey, C.L. and Gilbert, K.V. 1974. Temperature-dependent cooking toughness in beef. *J. Sci. Food Agric.* 25:931.
- Davey, C.L. and Gilbert, K.V. 1974a. Carcass posture and tenderness in frozen lambs. *J. Sci. Food* 25:923.
- Davey, C.L. and Gilbert, K.V. 1975. Cold shortening capacity and beef muscle growth. *J. Sci. Food Agric.* 26:755.
- Davey, C.L. and Graahuis, A.E. 1976. Structural changes in beef muscle during aging. *J. Sci. Food Agric.* 26:301.
- Davey, C.L., Niederer, A.F. and Graahuis, A.E. 1976. Effects of aging and cooking on the tenderness of beef muscle. *J. Sci. Food Agric.* 26:251.
- Davis, G.W., Dutson, T.R., Smith, G.C. and Carpenter, Z.L. 1980. Fragmentation procedure for bovine longissimus muscle as an index of cooked steak tenderness. *J. Food Sci.* 45:880.
- Dayton, W.R., Goll, D.E., Stromer, M.H., Reville, W.S., Zeece, M.G. and Robson, R.M. 1975. Some properties of a calcium-activated protease that may be involved in myofibrillar protein turnover. In: *Cold Spring Harbor Conferences on Cell Proliferative*, Vol. 2 p. 551. E. Reich, D.B. Rifkin and E. Shaw, eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

- Dayton, W.R., Goll, D.E., Zeece, M.G., Robson, R.M., Reville, J. 1976. A  $\text{Ca}^{2+}$  activated protease possibly involved in myofibrillar protein turnover. Purification of porcine muscle. *Biochem.* 15:2150.
- Deatherage, F.E. 1957. Basic chemical consideration regarding the tenderness of meat. p. 25. in *Proceedings of the 10th Reciprocal Meat Conference*. Chicago, IL.
- Dennis, J.E., Shimizu, T., Reinach, F.C. and Fischman, D.A. 1984. Localization of C-protein isoforms in chicken skeletal muscle: ultrasound detection using monoclonal antibodies. *J. Cell. Biol.* 98:1514.
- Dransfield, E. 1993. Modelling postmortem tenderization - IV: Role of calpains and calpastatin in conditioning. *Meat Sci.* 34:217.
- Dransfield, E. and Etherington, D.J. 1988. Electrical stimulation and meat texture – a response to preliminary reports on the toughness effect of electrical stimulation. *Meat Sci.* 24:227.
- Dransfield, E., Jones, R.C. D. and MacFie, H.J.H. 1980. Quantifying changes in tenderness during storage of beef. *Meat Sci.* 5:131.
- Dransfield, E., Wakefield, D.K. and Parkman, I.O. 1992. Modeling postmortem tenderization. I: Texture of electrically stimulated and non-stimulated beef. *Meat Sci.* 31:57.
- Dutson, T.R. 1983. Relationship of pH and temperature to disruption of specific muscle proteins and activity of lysosomal proteases. *J. Food Biochem.* 7:223.
- Dutson, T.R., Smith, G.C. and Carpenter, Z.L. 1982. Lysosomal enzyme distributor in electrically stimulated ovine muscle. *J. Food Sci.* 45:1097.
- Dutson, T.R. and Lawrie, R.A. 1974. Release of lysosomal enzymes during postmortem conditioning and their relationship to tenderness. *J. Food Technol.* 9:43.

- Dutson, T.R., Smith, G.C., Hostetler, R.L. and Carpenter, Z.L. 1975. Postmortem carcass temperature and beef tenderness. *J. Anim. Sci.* 41:289.
- Dziezak, J.D. 1990. Phosphates improve many foods. *Food Tech.* 44:80.
- Ebashi, S. and Ebashi, F. 1965.  $\alpha$ -actinin, a new structural protein from skeletal muscle. I. Preparation and action on actomyosin - ATP interaction. *J. Biochem.* 58:7.
- Ebashi, S. and Endo, M. 1968. Calcium ion and muscle contraction. *Prog. Biophys. Mol. Biol.* 18:123.
- Edmunds, T, Nagainis, P.A., Sathe, S.K., Thompson, V.F. and Goll, D.E. 1991. Comparison of the autolyzed and unautolyzed forms of u- and m-calpain from bovine skeletal muscle. *Biochem. Biophys. Acta.* 1077:197.
- El-Gharbawi, M. and Whitaker, J.R. 1963. Factors affecting enzymatic solubilization of beef proteins. *J. Food Sci.* 28:168.
- Etherington, D.J., Taylor, M.A.J. and Dransfield, E. 1987. Conditioning of meat from different species, relationship between tenderness and the levels of cathepsin B, cathepsin L, calpain 1, calpain 2 and B-glucuronidase. *Meat Sci.* 20:1.
- Fisher, C. and Hamm, R. 1980. Biochemical studies on fast glycolysing bovine muscle. *Meat Sci.* 4:41.
- Foegeding, E.A. and Ramsey, S.R. 1986. Effects of gums on low fat meat batters. *J. Food Sci.* 51:33.
- Foegeding, E.A. and Ramsey, S.R. 1987. Rheological and water holding properties of gelled meat batters containing iota carrageenan, kappa carrageenan or xanthan gum. *J. Food Sci.* 52:549.
- Furst, D.O., Nave, R., Osborn, M. and Weber, K. 1988. Repetitive titin epitopes with a 42 nm spacing coincide in relative position with known A-band striations also identified by major myosin-associated proteins. *J. Cell Sci.* 94:119.



- Geesink, G. 1993a. The role of ultimate pH in proteolysis and calpain/calpastatin activity in bovine muscles. p. 69-77. in G. Geesink, ed., Postmortem muscle proteolysis and beef tenderness with special reference to the action of the calpain/calpastatin system. Dekanen, Netherlands.
- Geesink, G. 1993b. The effects of calcium chloride and sodium chloride injection on meat quality. p. 109-120. in G. Geesink ed., Postmortem muscle proteolysis and beef tenderness with special reference to the action of the calpain/calpastatin system. Dekanen, Netherlands.
- Godsalve, E.W., Gordon, E.A. and Davis, H.T. 1977. Water loss rates and temperature profiles of dry cooked bovine muscle. J. Food Sci. 42:1038.
- Goll, D.E., Bray, R.W. and Hoekstra, W.G. 1964. Age associated changes in bovine muscle connective tissue. III. Rate of solubilization at 100°C. J. Food Sci. 29:622.
- Goll, D.E., Henderson, D.W. and Kline, E.A. 1964. Postmortem changes in physical and chemical properties of bovine muscle. J. Food Sci. 29:590.
- Goll, D.E., Kleese, W.C. and Szpacenko, A. 1989. Skeletal muscle proteases and protein turnover p. 141. in Animal growth regulation. Campion, D.R., Hausman, G.J., Martin R.J., eds., Phenom Press, New York.
- Goll, D.E., Otsuka, Y., Nagainis, P.A., Shannon, J.D., Sathe, S.K. and Mugurama, M. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7:137.
- Goll, D.E., Robson, R.M., Stromer, M.H. 1984. Skeletal muscle. p. 75 in M.J. Swenson, ed., Skeletal muscle, nervous system, temperature regulation, and special senses. Dukes physiology of domestic animals. 10th Edition Cornell University Press, Ithaca, NY.
- Goll, D.E., Taylor, R.G., Christiansen, J.A. and Thompson, V.F. 1991. Role of proteinases and protein turnover in muscle growth and meat

- quality. p. 25 in Proceedings of the 41st Reciprocal Meat Conference. Laramie, WY. National Livestock and Meat Board, Chicago, IL.
- Goll, D.E., Thompson, V.F., Taylor, R.G. and Zalewska, T. 1992. Is calpain activity regulated by membranes and autolysis or by calcium and calpastatin? *Bio Essays* 14:549.
- Grau, R. and Hamm, R. 1953. A simple method for the detection of hydration in muscle. *Naturwissenschaften*. 40:29.
- Grove, B.K., Korner, V., Lehner, C., Doetschman, T.C., Perriard, J.C. and Eppenberger, H.M. 1984. A new 185,000 dalton skeletal muscle protein detected by monoclonal antibodies. *J. Cell. Biol.* 98:518.
- Hainfeld, J.F., Wail, J.S. and Wang, K. 1988. Titin: quantitative mass measurements by scanning transmission electron microscopy and structural implications for the sarcomere matrix of skeletal muscle. *Febs Lett.* 234:145.
- Hamm, R. 1959. Biochemistry of meat hydration. p. 17 in Proceedings of the 11th Research Conference. American Meat Institute. Chicago, IL.
- Hamm, R. 1960. Biochemistry of meat hydration. *Adv. Food Res.* 10:355.
- Hamm, R. 1986. Functional Properties of the myofibrillar system and their measurements. In: *Muscle as Food. Food Science and Technology series of monographs.* Peter J. Bechtel, ed. Academic Press, New York.
- Hamm, R. and Grau, R. 1955. The effect of phosphates on the bound water of meat. *Dtsch. Lebensmitt. Rdsch.* 51:106.
- Hanson, L.J. 1972. Development of Armour tenderometer for tenderness evaluation of beef carcasses. *J. Texture Stud.* 3:146.
- Hawkins, R.R., Davis, G.W., Cable, J.K., Jr. and Ramsey, C.B. 1987. Fragmentation index as an early postmortem predictor of beef tenderness. *J. Anim. Sci.* 64:171.

- Hawrysh, Z.J., Shand, P.J., Wolfe, F.H., and Price, M.A. 1987. Studies of extra low voltage electrical stimulation of mature beef carcasses. *Meat Sci.* 21:121.
- Hearne, L.E., Penfield, M.P., and Goertz, G.E. 1978. Heating effects on bovine semitendinosus: Shear, muscle fiber measurements, and cooking losses. *J. Food Sci.* 43:10.
- Hellendorn, E.W. 1962. Water binding capacity of meat as affected by phosphates. 1. Influence of sodium chloride and phosphates on the water retention of comminuted meat at various pH values. *Food Technol.* 16:119.
- Herring, H.K., Cassens, R.G. and Briskey, E.J. 1965. Further studies on bovine tenderness as influenced by carcass position, sarcomere and fiber diameter. *J. Food Sci.* 30:1049.
- Hill, C. and Weber, K. 1986. Monoclonal antibodies distinguish titins from heart and skeletal muscle *J. Cell Biol.* 102:1099.
- Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. *J. Food Sci.* 31:161.
- Hofmann, K. 1975. A new way for determining water binding in beef: the kapillar-volumeter. *Fleischwirtschaft.* 55:25.
- Honikel, K.O. 1992. The biochemical basis of meat conditioning in new technologies for meat and meat products. (Smolders, Toldra, Flores and Prieto, eds.) *Ecceamst* p.124.
- Honikel, K.O., Fischer, C., Hamid, A. and Hamm, R. 1981a. Influence of postmortem changes in bovine muscle on the water holding capacity of beef. Postmortem storage of muscle at 20°C. *J. Food Sci.* 46:1.
- Honikel, K.O., Hamid, A., Fischer, C. and Hamm, R. 1981b. Influence of postmortem changes in bovine muscle on the water-holding capacity of beef. Postmortem storage of muscle at various temperatures between 0°C and 30°C. *J. Food Sci.* 46:23.

- Honikel, K.O., Kim, C.J., Hamm, R. and Roncales, P. 1986. Sarcomere shortening of pre-rigor muscles and its influence on drip loss. *Meat Sci.* 16:267.
- Honikel, K.O., Fischer, C. and Hamm, R. 1980. The effects of cooling on the properties of fresh slaughtered beef. *Fleischwirtschaft.* 60:1577.
- Honikel, K.O., Kim, C.J., Roncuaes, P. and Hamm, R. 1986. Sarcomere shortening of prerigor muscles and its influence on drip loss. *Meat Sci.* 16:267.
- Horowitz, R., Maruyama, K. and Podolsky, R.J. 1989. Elastic behavior of connectin filaments during thick filament movement in activated skeletal muscle. *J. Cell Biol.* 109:2169.
- Hostetler, R.L., Landmann, W.A., Link, B.A. and Fitzhugh, H.A., Jr. 1970. Influence of carcass position during rigor mortis on tenderness of beef muscles: Comparison of two treatments. *J. Anim. Sci.* 31:47.
- Hostetler, R.L., Link, B.A., Landmann, W.A. and Fitzhugh, H.A., Jr. 1972. Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles.
- Hu, D.H., Kimura, S. and Maruyama, K. 1986. Sodium dodecyl sulfate gel electrophoresis studies of connectin-like high molecular weight proteins of various types of vertebrate and invertebrate muscles. *J. Biochem.* 99:1485.
- Huff, E.J. and Parrish, F.C., Jr. 1993. Bovine longissimus muscle tenderness as affected by postmortem aging time, animal age and sex. *J. Food Sci.* 58:713.
- Huff-Lonergan, E.J., Beekman, D.D. and Parrish, F.C., Jr. 1994. Protein separation and analysis of certain skeletal muscle proteins: Principles and techniques. In *Protein Functionality in Food Systems*, (Ed) Hettiarachchy, N.S., Zeigler, G.R. p. 79-119. Marcel Dekker Inc., New York.

- Huff-Lonergan, E.J., Parrish, F.C., Jr. and Robson, R.M. 1994a. Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *J. Anim. Sci.* (submitted).
- Huxley, H.E. 1972 . Molecular basis of contraction in cross-striated muscles. In: *Structure and function of muscles.* (Ed) Bourne, G. Vol. 1. 2nd ed. p. 89. Academic Press, New York.
- Huxley, H.E. 1958. The contraction of muscle. *Scientific American.* 19:3.
- Huxley, H.E. 1986. Comments on quantal mechanisms in cardiac contraction. *Circ. Res.* 59:9.
- Huxley, H.E. and Hanson, J. 1954. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature.* 173:973.
- Huxley, H.E., Faruqi, A.R. and Whytock, S. 1987. Correlation between x-ray diffraction studies on contracting muscles and electron microscope observations using rapid freezing techniques. *Biophys. J.* 51:5.
- Inomata, M., Hayashi, M., Nakamora, M., Imahori, K. and Kawashima, S. 1985. Hydrolytic and autolytic behavior of two forms of calcium activated neutral protease (CANP). *J. Biochem.* 98:407.
- Jakobsson, B. and Bengtsson, N. 1973. Freezing of raw beef: Influence of aging, freezing rate and cooking method on quality and yield. *J. Food Sci.* 38:560.
- Jauregui, C.A., Regenstein, J.M. and Baker, R.C. 1981. A simple centrifuged method for measuring expressable moisture, A water binding property of muscle foods. *J. Food Sci.* 46:1271.
- Jeremiah, L.E., Tony, A.K.W. and Gibson, L.L. 1991. The usefulness of muscle color and pH for separating beef carcasses into tenderness groups. *Meat Sci.* 30:97.

- Jin, J.P. and Wang, K. 1991. Nebulin as a giant actin binding template protein in skeletal muscle sarcomere. Interaction of actin and cloned human nebulin fragments. FEBS Lett. 281:93.
- Johnson, P.G. and Civille, G.V. 1986. Standard lexicon of meat WOF descriptors. J. Sensory Stud. 1:99.
- Kang, C.K. and Rice, E.E. 1970. Degradation of various meat fractions by tenderizing enzymes. J. Food Sci. 35:563.
- Kapprell, H.P. and Goll, D.E. 1989. Effect of  $\text{Ca}^{2+}$  on binding of the calpains to calpastatin. J. Biol. Chem. 264:17888.
- Kastner, C.L. 1983. Optimal heat processing systems for beef. Food Tech. 37:96.
- Kastner, C.L., Henrickson, R.L. and Morrison, R.D. 1973. Characteristics of hot boned bovine muscle. J. Anim. Sci. 36:484.
- Katoh, K. 1981. An improved method for determining water holding capacity of meat. Jpn. J. Zootech. Sci. 52:139.
- Kauffman, R.G., Eikelenboom, G., Van der Wal, P.G., Merkus, G. and Zaar, M. 1986. The use of filter paper to estimate drip loss of porcine musculature. Meat Sci. 18:191.
- Knight, P. and Parsons, N. 1988. Action of NaCl and polyphosphates in meat processing: responses of myofibrils to concentrated salt solutions. Meat Sci. 24:275.
- Koohmaraie, M. 1988. The role of endogenous protease in meat tenderness. p. 89 in Proceedings of the 41st Reciprocal Meat Conference. Laramie, WY. National Livestock and Meat Board, Chicago, IL.
- Koohmaraie, M. 1992. Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle m-calpain. J. Anim. Sci. 70:3071.

- Koohmaraie, M. 1992. Role of neutral proteases in postmortem muscle protein degradation and meat tenderness. p. 63 in Proceedings of the 45th Reciprocal Meat Conference. Fort Collins, CO. National Livestock and Meat Board, Chicago, IL.
- Koohmaraie, M., Babiker, A.S., Merkel, R.A. and Dutson, T.R. 1988a. The role of  $\text{Ca}^{2+}$  dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. 53:1253.
- Koohmaraie, M., Babiker, A.S., Schroeder, A.L., Merkel, R.A. and Dutson, T.R. 1988b. Acceleration of postmortem tenderization in ovine carcasses through activation of  $\text{Ca}^{2+}$  dependent protease. J. Food Sci. 53:1638.
- Koohmaraie, M., Crouse, J.D. and Mersmann, H.J. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: effect of concentration and ionic strength. J. Anim. Sci. 67:934.
- Koohmaraie, M., Kennick, W.H., Elgasim, E.A. and Anglemier, A.F. 1984. Effects of postmortem storage on muscle protein degradation: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49:292.
- Koohmaraie, M., Seideman, S.E., Schollmeyer, J.E., Dutson, T.R. and Crouse, J.D. 1987. Effects of postmortem storage on  $\text{Ca}^{2+}$  dependent proteases, their inhibitors and myofibrillar fragmentation. Meat Sci. 19:187.
- Kramer, A., Hamlid, K., Guyer, R.B. and Rogers, H.P. 1951. New shear press predicts quality of canned lima beans. Food Engin. 23:112.
- Kruggel, W.G., Field, R.A., and Miller, G.J. 1970. Physical and chemical properties of epimysial acid-soluble collagen from meats of ranging tenderness. J. Food Sci. 35:106.
- Kuhne, W. 1964. Experiments observing protoplasm and contractility. ed Engelmann, D. Leipzig, Germany.

- Kumamoto, T., Kleese, W.C., Cong, J., Goll, D.E., Pierce, P.R. and Allen, R.E. 1992. Localization of the  $\text{Ca}^{2+}$  dependent proteases and their inhibitor in normal, fasted and innervated rat skeletal muscle. *The Anat. Record.* 232:60.
- Kurzbam, G.P. and Wang, K. 1988. Giant polypeptides of skeletal muscle titin: sedimentation equilibrium in guanidine hydrochloride. *Biochem Biophys. Res. Commun.* 150:1155.
- Labeit, S., Barlow, D.P., Gavel, M., Gibson, T., Holt, J., Hsieh, C.L., Francke, U., Leonard, K., Wardle, J., Whiting, A. and Trinick, Jr. 1990. A regular pattern of two types of 100 residue motif in the sequence of titin. *Nature* 345:273.
- Laemmli, V.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680.
- Lawrie, R.A., Sharp, J.G., Bendell, J.R. and Coleby, B. 1961. Treatments of meats with ionising radiations. VIII. pH, water-binding capacity and proteolysis of irradiated raw beef and pork during storage, and the ATP-ase activity of irradiated rabbit muscle. *J. Sci. Food Agric.* 12:742.
- Lehman, K.B. 1907. Studies on the toughness of meat and its origin. *Arch. Hyg.* 63:134.
- Light, N.D., Champion, A.E., Voyle, C. and Bailey, A.J. 1985. The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. *Meat Sci.* 13:137.
- Locker, R.H. 1959. Striation patterns of ox muscle in rigor mortis. *J. Biophys. Biochem. Cytol.* 6:419.
- Locker, R.H. 1960. Degree of muscular contraction as a factor in tenderness of beef. *Food Res.* 25:304.
- Locker, R.H. 1982. A new theory of tenderness in meat, based on gap filaments. p. 92 in *Proceedings of the 35th Reciprocal Meat Conference*. Blacksburg, VA. National Livestock and Meat Board, Chicago, IL.



- Locker, R.H. 1984. The role of gap filaments in muscle and meat. *Food Microstrut.* 3:17.
- Locker, R.H. 1987. The non-sliding filaments of the sarcomere. *Meat Sci.* 20:217.
- Locker, R.H. and Daines, G.J. 1975. Rigor mortis in beef sternomandibularis muscle at 37°C. *J. Sci. Food Agric.* 26:1721.
- Locker, R.H. and Daines, G.J. 1980. Gap filaments – the third set of filaments in the myofibril. p. 43 In *Fibrous proteins: scientific, industrial and medical aspects*. Parry, D.A.D. and Creamer, L.K., eds. Vol. 2. Academic Press, London.
- Locker, R.H. and Hagyard, C.J. 1963. A cold shortening effect in beef muscles. *J. Sci. Food Agric.* 14:787.
- Locker, R.H. and Leet, N.G. 1975. Histology of highly-stretched beef muscle. I. The fine structure of grossly stretched single fibers. *J. Ultrastructure Res.* 52:64.
- Locker, R.H. and Leet, N.G. 1976a. Histology of highly-stretched beef muscle. II. Further evidence on the location and nature of gap filaments. *J. Ultrastructure Res.* 55:157.
- Locker, R.H. and Leet, N.G. 1976b. Histology of highly-stretched beef muscle. IV. Evidence for movement of gap filaments through the Z-line, using the N<sub>2</sub>-line and M-line as markers. *J. Ultrastructure Res.* 56:31.
- Locker, R.H. and Wild, D.J.C. 1986. A comparative study of high molecular weight proteins in various types of muscle across the animal kingdom. *J. Biochem.* 99:1473.
- Locker, R.H., Daines, G.J. and Leet, N.G. 1976. Histology of highly-stretched beef muscle. III. Abnormal contraction patterns in ox muscle produced by overstretching during pre-rigor blending. *J. Ultrastructure Res.* 5:173.

- Locker, R.H., Daines, G.J., Carse, W.a. and Leet, N.G. 1977. Meat tenderness and the gap filaments. *Meat Sci.* 1:87.
- Lusby, M.L., Ridpath, J.F., Parrish, F.C., Jr., and Robson, R.M. 1983. Effect of postmortem storage on degradation of the myofibrillar protein titin in bovine longissimus muscle. *J. Food Sci.* 48:1787.
- MacBride, M.A. and Parrish, F.C., Jr. 1977. The 30,000 Dalton component of tender bovine longissimus muscle. *J. Food Sci.* 42:1627.
- Mackintosh, D.L., Hall, J.L. and Vail, G.E. 1936. Some observations pertaining to tenderness of meat. p. 285 in *Proceedings of the American Society of Animal Production.* vol. 29.
- Marsh, B.B. 1983. Effects of early postmortem muscle pH and temperature on meat tenderness. p. 131. in *Proceedings of the 36th Reciprocal Meat Conference.* Fargo, N.D. National Livestock and Meat Board, Chicago, IL.
- Marsh, B.B. 1985. Electrical stimulation. In *Advances in Meat Science* 1: AVI publishers, Westport, CT. p. 277.
- Marsh, B.B. and Leet, N.G. 1966. Studies in meat tenderness. III. The effects of cold shortening on tenderness. *J. Food Sci.* 31:450.
- Marsh, B.B. and Thompson, J.F. 1958. Rigor mortis and thaw rigor in lambs. *J. Sci. Food Agric.* 9:414.
- Marsh, B.B., Ringkob, T.P., Russell, R.L., Swartz, D.O. and Pagel, L.A. 1988. Mechanisms and strategies for improving meat tenderness. p. 113 in *Proceedings of the 41st Reciprocal Meat Conference.* Laramie, WY, National Livestock and Meat Board, Chicago, IL.
- Marsh, B.B., Ringkob, T.P., Russell, R.L., Swartz, D.R. and Pajel, L.A. 1987. Effects of early postmortem glycolytic rate on beef tenderness. *Meat Sci.* 21:241.
- Marsh, B.B., Woodhams, P.R. and Leef, N.G. 1968. Studies in meat tenderness. 5. The effects on tenderness of carcass cooling and freezing before the completion of rigor mortis. *J. Food Sci.* 33:12.

- Maruyama, K. 1976. Connectin, an elastic protein of myofibrils. J. Biochem. 80:405.
- Maruyama, K. 1985. Myofibrillar and cytoskeletal proteins of vertebrate striated muscle. p. 25. In: Developments in Meat Science vol. 3. Lawrie, R., ed. Elsevier Applied Sci. Pub. London and New York.
- Maruyama, K. 1986. Connectin, an elastic filamentous protein of striated muscle. Int. Rev. Cytol. 104:81.
- Maruyama, K. and Ebashi, S. 1965.  $\alpha$ -actinin, a new structural protein from striated muscle. II. Action on actin. J. Biochem. 58:13.
- Maruyama, K., Kimura, S., Ohashi, K., Kuwano, K. 1981. Connectin, an elastic protein of muscle identification of titin with connectin. J. Biochem. 89:701.
- Maruyama, K., Kimura, S., Yoshidomi, H., Sawada, H. and Kikuchi, M. 1984. Molecular size and shape of  $\beta$ -connectin, an elastic protein of striated muscle. J. Biochem. 95:1423.
- Maruyama, R., Kemura, M., Kimura, S., Ohashi, K., Suzuki, K. and Katonuma, N. 1981. Connectin, an elastic protein of muscle. Effects of proteolytic enzyme in situ. J. Biochem. 89:711.
- Matsuura, T., Kimura, S., Ohtsuka, S., Maruyama, K. 1991. Isolation and characterization of 1,200 Kda peptide of  $\alpha$ -connectin. J. Biochem. 110:474.
- McBee, J.L., Jr. and Wiles, J.A. 1967. Influence of marbling and carcass grade on the physical and chemical characteristics of beef. J. Anim. Sci. 26:701.
- McClain, P.E. and Mullins, A.M. 1969. Relationship of water binding and pH to tenderness of bovine muscle. J. Anim. Sci. 29:268.
- McGinnis, J.P., Papa, L.M., and Fletcher, D.L. 1989. Influence of sodium pentobarbital on pH, sarcomere length, and muscle tenderness of broiler pectoralis major muscle. Poultry Sci. 68:244.

- McKeith, F.K., DeVol, D.L., Miles, R.S., Bechtel, P.J. and Carr, T.R. 1985. Chemical and sensory properties of thirteen major beef muscles. *J. Food Sci.* 50:869.
- McKeith, F.K., Savell, J.W. and Smith, G.C. 1981. Tenderness improvement of the major muscles of the beef carcasses by electrical stimulation. *J. Food Sci.* 46:1774.
- McKeith, F.K., Smith, G.C., Savell, J.W., Dutson, T.R., Carpenter, Z.L. and Hammons, D.R. 1981. Effects of certain electrical stimulation parameters on quality and palatability of beef. *J. Food Sci.* 46:13.
- Medeiros, L.C., Field, R.A., Menkhaus, D.J. and Russell, W.C. 1987. Evaluation of range-grazed and concentrate-fed beef by a trained sensory panel, a household panel and a laboratory test market group. *J. Sensory Stud.* 2:259.
- Miller, M.F., Cross, H.R., Cruse, J.D. and Jenkins, T.G. 1987. Effect of energy intake on collagen characteristics and muscle quality of mature cows. *Meat Sci.* 21:287.
- Miller, W.O., Suffle, R.L. and Zirckle, S.B. 1968. Factors which influence the water holding capacity of various types of meat. *Food Tech.* 22:89.
- Miyada, D.S. and Tappel, A.L. 1956a. Meat tenderization. I. Two mechanical devices for measuring texture. *Food Tech.* 10:142.
- Miyada, D.S. and Tappel, A.L. 1956b. The hydrolysis of beef proteins by various proteolytic enzymes. *Food Res.* 21:217.
- Moos, C., Mason, C.M., Bestman, J.M., Feng, I.N.M. and Dubon, J.H. 1981. The binding of skeletal muscle C-protein to F-actin and its relation the interaction of actin with myosin subfragment-1. *J. Mol. Biol.* 124:571.
- Morel, J.E. and Merah, Z. 1992. Muscle contraction and in vitro movement: role of actin? *J. Mus. Cell. Motil.* 13:5.

- Morgan, J.B., Miller, R.K., Mendez, F.M., Hale, D.S. and Savell, J.W. 1991a. Using calcium chloride injection to improve tenderness of beef from mature cows. *J. Anim. Sci.* 69:4469.
- Morgan, J.B., Savell, J.W., Hale, D.S., Miller, R.K., Griffin, D.B., Cross, H.R. and Shackelford, S.D. 1991b. National beef tenderness survey. *J. Anim. Sci.* 69:3274.
- Murakami, V. and Uchida, K. 1985. Contents of myofibrillar proteins in cardiac, skeletal and smooth muscles. *J. Biochem.* 98:187.
- Murray, J.M. and A. Webber. 1974. The cooperative action of muscle proteins. *Scientific American.* 230:58.
- Nakamura, M., Inomata, M., Imajoh, S., Suzuki, K. and Kawashima, S. 1989. Fragmentation of an endogenous inhibitor upon complex formation with high and low  $\text{Ca}^{2+}$  requiring forms of calcium-activated neutral proteases. *Biochem.* 28:449.
- Nave, R., Furst, D.O. and Weber, K. 1989. Visualization of the polarity of isolated titin molecules: A single globular head on a long thin rod as the M-band anchoring domain. *J. Cell Biol.* 109:2177.
- Nave, R., Furst, D.O. and Weber, K. 1990. Interaction of  $\alpha$ -actinin and nebulin in vitro. *FEBS Lett.* 269:163.
- Ockerman, H.W. 1991. *Food Science Sourcebook*, 2nd ed. p. 532. Van Nostrand Reinhold, New York.
- Ockerman, H.W. and Wu, Y.C. 1990. Hot-boning, tumbling, salt and chopping temperature effects on cooking yield and acceptability of emulsion-type pork sausage. *J. Food Sci.* 55:1255.
- Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Sci.* 8:245.
- Ohashi, K. and Maruyama, K. 1989. Refined purification and characterization of Z-proteins. *J. Biochem.* 106:110.

- Olson, D.G. and Parrish, F.C., Jr. 1977. Relationship of myofibril fragmentation index to measures of beefsteak tenderness. *J. Food Sci.* 42:506.
- Olson, D.G., Parrish, F.C., Jr. and Stromer, M.H. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* 41:1036.
- Olson, D.G., Parrish, F.C., Jr., Dayton, W.R. and Goll, D.E. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. *J. Food Sci.* 42:117.
- Orcutt, M.W. and Dutson, T.R. 1985. Postmortem degradation of gap filaments at different postmortem pH's and temperatures. *Meat Sci.* 14:221.
- Ouali, A. 1990. Meat tenderization: possible causes and mechanisms. A review. *J. Muscle Foods.* 1:129.
- Ouali, A., Garrel, N., Obled, A., Deval, C., Valin, C. and Penny, I.F. 1982. Comparative action of cathepsins D, B, H and L, and of a new lysosomal cysteine proteinase on rabbit myofibrils. *Meat Sci.* 19:83.
- Ouali, A., Olded, A., Cottin, P., Merduli, N., Ducastaing, A. and Valin, C. 1983. Comparative effects of postmortem storage and low-calcium requiring neutral proteinase on bovine and rabbit myofibrillar proteins. *J. Sci. Food Agric.* 34:466.
- Palladino, D.K. and Ball, H.R., Jr. 1979. Effects of selected inorganic salts on certain tenderness characteristics of spent hens. *J. Food Sci.* 44:322.
- Parrish, F.C., Jr. and M.L. Lusby. 1983. An overview of a symposium on the fundamental properties of muscle proteins important in meat science. *J. Food Biochem.* 7:125.
- Parrish, F.C., Jr., Olson, D.G., Miner, B.E., Young, R.B., Snell, R.L. 1973. Relationship of tenderness measurements made by the Armour tenderometer to certain objective, subjective and organoleptic properties of bovine muscle. *J. Food Sci.* 38:1284.

- Parrish, F.C., Jr., Paterson, B.C. and Paxhia, J.M. 1988. Titin: a myofibrillar/cytoskeletal protein of gigantic proportions. p. 15. in Proceedure of the 41st Reciprocal Meat Conference. Laramie, WY. National Livestock and Meat Board, Chicago, IL.
- Parrish, F.C., Jr., Selvig, C.J., Culler, R.D. and Zeece, M.G. 1981. CAF activity, calcium concentration, and the 30,000-dalton component of tough and tender bovine longissimus muscle. *J. Food Sci.* 46:308.
- Parrish, F.C., Jr., Vandell, C.J. and Culler, R.D. 1979. Effects of maturity and marbling on the myofibril fragmentation index of bovine longissimus muscle. *J. Food Sci.* 44:1668.
- Parrish, F.C., Jr., Young, R.B., Miner, B.E. and Anderson, L.D. 1973. Effect of postmortem conditions on certain chemical, morphological and organoleptic properties of bovine muscle. *J. Food Sci.* 38:690.
- Parsons, N. and Knight, P. 1990. Origin of variable extraction of myosin from myofibrils treated with salt and pyrophosphate. *J. Sci. Food Agric.* 51:71.
- Paterson, B.C. and Parrish, F.C., Jr. 1986. A sensory panel and chemical analysis of certain beef chuck muscles. *J. Food Sci.* 51:876.
- Paterson, B.C. and Parrish, F.C., Jr. 1987. SDS-Page conditions for detection of titin and nebulin in tender and tough bovine muscles. *J. Food Sci.* 52:509.
- Paterson, B.C., Parrish, F.C., Jr. and Stromer, M.H. 1988. Effects of salt and pyrophosphate on the physcial and chemical properties of beef muscles. *J. Food Sci.* 53:1258.
- Paxhia, J.M. and Parrish, F.C., Jr. 1988. Effects of postmortem storage on titin and nebulin in pork and poultry light and dark muscles. *J. Food Sci.* 53:1599.
- Pepe, F. and Prockel, B. 1975. The myosin filament. III. C-protein. *J. Molec. Biol.* 99:609.

- Pette, D. and R.S. Staron. 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev. Physiol. Biochem. Pharmacol.* vol. 116:15.
- Pike, M.M., Ringkob, T.P., Beekman, D.D., Koh, Y.O. and Gerthoffer, W.T. 1993. Quadratic relationship between early postmortem glycolytic rate and beef tenderness. *Meat Sci.* 34:13.
- Pollack, G.H. 1988. Cross-bridge theory: Do not disturb! *J. Mol. Cell. Cardiol.* 20:563.
- Powell, V.H. 1991. Quality of beef loin steaks as influenced by animal age, electrical stimulation and aging. *Meat Sci.* 30:195.
- Powell, V.H., Dickinson, R.F., McPhail, N.G., Bouton, P.E., Harris, P.V. 1984. Evaluation of extra low voltage electrical stimulation systems for bovine carcasses. *J. Food Sci.* 49:363.
- Proctor, B.E., Davison, S., Molecki, G.J., and Welch, M. 1955. A recording strain gauge denture tenderometer for foods. I. Instrument evaluation and initial tests. *Food Tech.* 9:471.
- Purchas, R.W. 1990. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. *Meat Sci.* 27:129.
- Purslow, P.P. 1985. The physical basis of meat texture: observations on the fracture behavior of cooked bovine semitendinosus. *Meat Sci.* 12:39.
- Radomyski, T. 1994. Personal communication. Iowa State University, Ames, IA.
- Rainey, B.A. 1986. Importance of reference standards in training panelists. *J. Sensory Stud.* 1:149.
- Rajalakshami, D., Dhanaraj, S., Chand, N. and Govinderajan, V.S. 1989. Descriptive quality analysis of mutton. *J. Sensory Stud.* 2:93.



- Ramsbottom, J.M., Strandine, E.J. and Koonz, C.H. 1945. Comparative tenderness of representative beef muscles. *Food Research*. 10:497.
- Rao, M.V., Gault, N.F.S. and Kennedy, S. 1989. Variations in water-holding capacity due to changes in the fiber diameter, sarcomere length, and connective tissue morphology of some beef muscles under acidic conditions below the ultimate pH. *Meat Sci*. 26:19.
- Reagan, J.O., Dutson, T.R., Carpenter, Z.L. and Smith, G.C. 1975. Muscle fragmentation index for predicting cooked beef tenderness. *J. Food Sci*. 40:1093.
- Regenstein, J.M. and Rank Stamm, J. 1979a. The effect of sodium polyphosphates and of divalent cations on the water holding capacity of pre- and post-rigor chicken breast muscle. *J. Food Biochem*. 3:213.
- Regenstein, J.M. and Rank Stamm, J. 1979b. A comparison of the water holding capacity of pre- and post-rigor chicken, trout and lobster muscle in the presence of polyphosphates and divalent cations. *J. Food Biochem*. 3:223.
- Regenstein, J.M., Gorimar, T.S. and Sherbon, J.W. 1979. Measuring the water holding capacity of natural actomyosin from chicken breast muscle in the presence of pyrophosphate and divalent cations. *J. Food Biochem*. 3:205.
- Remedios, C.G. and Gilmour, D. 1978. Is there a third type of filament in striated muscle? *J. Biochem*. 84:235.
- Riette, L.J.M. and Smulders, F.J.M. 1989. The effect of electrical stimulation, time of boning and high temperature conditioning on sensory quality traits of porcine longissimus dorsi muscle. *Meat Sci*. 25:113.
- Ringkob, T.P., Marsh, B.B. and Greaser, M.L. 1988. Change in titin position in postmortem bovine muscle. *J. Food Sci*. 53:276.
- Risvik, E. 1986. Sensory evaluation of irradiated beef and bacon. *Sensory Stud*. 1:109.

- Robson, R.M. and Huiatt, T.W. 1983. Roles of the cytoskeletal proteins desmin, titin and nebulin in muscle. p. 116 in Proceedings of the 36th Reciprocal Meat Conference. Fargo, ND. National Livestock and Meat Board, Chicago, IL.
- Robson, R.M., Huiatt, T.W. and Parrish, F.C., Jr. 1991. Biochemical and structure properties of titin, nebulin and intermediate filaments in muscle. p. 7. in Proceedings of the 44th Reciprocal Meat Conference. Manhattan, KS. National Livestock and Meat Board, Chicago, IL.
- Robson, R.M., O'Shea, J.M., Rathbun, W.E., LaSall, F., Schreiner, P.J., Kasang, L.E., Stromer, M.H., Lusby, M.L., Ridpath, J.F., Pang, Y.Y., Evans, R.R., Zeece, M.G., Parrish, F.C., Jr., and Huiatt, T.W. 1984. Role of new cytoskeletal elements in maintenance of muscle integrity. J. Food Biochem. 8:1.
- Rolan, T.L., Davis, G.W., Seideman, S.C., Wheeler, T.L. and Miller, M.F. 1988. Effects of blade tenderization and proteolytic enzyme on restructured steaks from beef bullock chucks. J. Food Sci. 53:1062.
- Rome, E., Offer, G. and Pepe, F. 1973. X-ray diffraction of muscle labelled with antibody to C-protein. Nature. 244:152.
- Sanderson, M. and Vail, G.E. 1963. A method for determing press fluid in cooked beef. J. Food Sci. 28:596.
- SAS. 1988. SAS/STAT User's guide. Release 6.03. SAS Inst., Cary, NC.
- Satorius, M.J. and Child, A.M. 1938. Effect of coagulation on press fluid, shear force, muscle cell diameter and composition of beef muscle. Food Research. 3:619.
- Savell, J.W. 1979. Update: Industry acceptance of electrical stimulation. p. 113. in Proceedures of the 32nd. Reciprocal Meat Conference. Brookings, SD. National Livestock and Meat Board, Chicago, IL.
- Savell, J.W., Dutson, T.R., Smith, G.C., and Carpenter, Z.L. 1978. Structural changes in electrically stimulated beef muscle. J. Food Sci. 43:1606.

- Savell, J.W., Smith, G.C., Carpenter, Z.L. and Parrish, F.C., Jr. 1979. Influence of electrical stimulation on certain characteristics of heavy weight beef carcasses. *J. Food Sci.* 44:911.
- Schutt, C.E. and Lindberg, U. 1992. Actin as the generator of tension during muscle contraction. *Proc. Natl. Acad. Sci. USA.* 89:319.
- Seideman, S.C., Koohmaraie, M. and J.D. Crouse. 1987. Factors associated with tenderness in young beef. *Meat Sci.* 20:281.
- Shaub, M.C. and Perry, S.V. 1969. The relaxing protein system of striated muscle. *Biochem J.* 115:993.
- Shults, G.W., Russell, D.R. and Wierbicki, E. 1972. Effect of condensed phosphates on pH, swelling and water-holding capacity of beef. *J. Food Sci.* 37:860.
- Sjostrand, F.S. 1962. The connections between A and I-band filaments in striated frog muscle. *J. Ultrastructure Res.* 7:225.
- Smith, G.C., Carpenter, Z.L. and King, G.T. 1969. Considerations for beef tenderness evaluations. *J. Food Sci.* 34:612.
- Smith, M.E., Kastner, C.L., Hunt, M.C., Kropf, D.H. and Allen, D.M. 1979. Elevated conditioning temperature effects on beef carcasses from four nutritional regimes. *J. Food Sci.* 44:158.
- Smulders, F.J.M., Marsh, B.B., Swartz, D.R., Russell, R.L. and Hoenecke, M.E. 1990. Beef tenderness and sarcomere length. *Meat Sci.* 28:349.
- Sorinmade, S.O., Cross, H.R., Ono, K., and Wergin, W.P. 1982. Mechanisms of ultrastructural changes in electrically stimulated beef longissimus muscle. *Meat Sci.* 6:71.
- Sperring, D.D., Platt, W.T. and Hiner, R.L. 1959. Tenderness in beef muscle as measured by pressure. *Food Tech.* 13:155.
- Squire, J.M. 1986. Myosin molecules. In: *Muscle design diversity and disease.* p. 116, Ch. 4. (Ed) King, J. B. Cummings publ., Menlo Park, CA.

- St. Angelo, A.J., Koohmaraie, M., Crippen, K.L. and Crouse, J. 1991. Acceleration of tenderization/inhibition of warmed over flavor by calcium chloride antioxidant infusion into lamb carcasses. *J. Food Sci.* 56:359.
- Stanton, C. and Light N. 1990. The effects of conditioning on meat collagen: Part 3—evidence for proteolytic damage to endomysial collagen after conditioning. *Meat Sci.* 27:41.
- Starr, R. and Offer, G. 1971. Poly peptide chains of intermediate molecular weight in myosin preparations. *Febs Lett.* 15:40.
- Starr, R. and Offer, G. 1978. The interaction of C-protein with heavy meromyosin and subfragment-2. *Biochem. J.* 171:813.
- Stiffler, D.M., Smith, G.C., Savell, J.W., Dutson, T.R., Griffin, C.L. and Orcutt, M.W. 1984. Comparison of the effects of high and low voltage electrical stimulation on quality indicating characteristics of beef carcasses. *J. Food Sci.* 49:863.
- Straub, F.B. 1942. Master Thesis, University of Szeyed II, 3-15.
- Studier, R.W. 1973. Analysis of bacteriophage T7 early RNA's and proteins on slab gels. *J. Mol. Biol.* 79:237.
- Swift, C.E. and Ellis, R. 1956. The action of phosphates in sausage products. 1. Factors affecting the water retention of phosphate treated ground meat. *Food Technol.* 13:546.
- Szent-Gyorgyi, A.C. 1953. Meromyosins, the subunits of myosin. *Arch. Biochem. Biophys.* 42:305.
- Takahashi, G., Wang, S.M., Lochner, J.V., and Marsh, B.B. 1987. Effects of 2-Hz and 60Hz electrical stimulation on the microstructure of beef. *Meat Sci.* 19:65.
- Takahashi, K., Hattori, A., Tatsumi, R. and Takai, K. 1992. Calcium-induced splitting of connectin filaments into  $\beta$ -connectin and a 1,200 Kda subfragment. *J. Biochem.* 111:778.

- Tan, K.O., Sater, G.R., Myers, A.M., Robson, R.M., Huiatt, T.W. 1993. Molecular characterization of avian muscle titin. *J. Biol. Chem.* 268:22900.
- Tatsumi, R. and Takahashi, K. 1992. Calcium induced fragmentation of skeletal muscle nebulin filaments. *J. Biochem.* 112:775.
- Taylor, M.A. and Etherington, D.J. 1991. The solubilization of myofibrillar proteins by calcium ions. *Meat Sci.* 29:211.
- Tresseler, D.K., Birdseye, C. and Murray, W.T. 1932. Tenderness of meat. I. Determination of relative tenderness of chilled quick frozen beef. *Industrial and Engineering Chemistry.* 24:242.
- Trinick, J. 1992. Molecular rulers in muscle? *Curr. Biol.* 2:75.
- Trinick, J. and Lowey, S. 1977. M-protein from chicken pectoralis muscle: isolation and characterization. *J. Mol. Biol.* 113:343.
- Trinick, J., Knight, P. and Whiting, A. 1984. Purification and properties of native titin. *J. Mol. Biol.* 180:331.
- Trout, G.R. 1988. Techniques for measuring water-binding capacity in muscle foods – A review of methodology. *Meat Sci.* 23:235.
- Trout, G.R. and Schmidt, G.R. 1983. Utilization of phosphates in meat products. p. 24. in *Proceedures of the 36th Reciprocal Meat Conference*. Fargo, ND. National Livestock and Meat Board, Chicago, IL.
- Troy, D.J., Tarrant, V.P. and Harrington, M.G. 1986. Electrophoretic analysis of changes in beef myofibrillar proteins during the early postmortem period. *Biochem. Soc. Trans.* 14:436.
- Tsen, C.C. and Tappel. 1959. Meat tenderization. III. Hydrolysis of actomyosin, actin and collagen by papain. *Food Res.* 24:362.
- Turner, D.C., Wallimann, T., and Eppenberger, H.M. 1973. A protein that binds specifically to the M-line of skeletal muscle is identified as

- the muscle form of creatine kinase. *Proc. Natl. Acad. Sci. USA.* 70:702.
- Ulrick, W.C., Toselli, P.A., Chase, D., Dasse, K. 1977. Are there extensions of thick filaments to the Z line in vertebrate and invertebrate striated muscle? *J. Ultrastructure Res.* 60:263.
- Unruh, J.A., Kastner, C.L., Kropf, D.H., Dikeman, M.E., and Hunt, M.C. 1984. Effects of low voltage electrical stimulation during exsanguination on characteristics of beef longissimus and semimembranosus muscles. p. 181. in *Proceedures of the 37th Reciprocal Meat Conference.* Lubbock, TX. National Livestock and Meat Board, Chicago, IL.
- Uytterhaegen, L. Claeys and Demeyer, D. 1994. Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. *J. Anim. Sci.* 72:1209.
- Volodkevich, N.N. 1938. Apparatus for measurements of chewing; resistances or tenderness of food stuffs. *Food Research.* 3:221.
- Wang, H., Wier, C.E., Birkner, M.L., and Ginger, B. 1957. The influence of enzyme tenderizer on the structure and tenderness of beef. *Proc. of 9th Rec. Conf.*, p. 80. Amer. Meat Inst., Univ. of Chicago, Chicago, IL.
- Wang, K. 1981. Nebulin, a giant protein component of N<sub>2</sub>-line of striated muscle. *J. Cell Biol.* 91:355a.
- Wang, K. and Greaser, M.L. 1985. Immunocytochemical studies using a monoclonal antibody to bovine cardiac titin on intact and extracted myofibrils. *J. Muscle Res. Cell Motil.* 6:293.
- Wang, K. and Williamson, C.L. 1980. Identification of an N<sub>2</sub>-line protein of striated muscle. *Proc. Natl. Acad. Sci. USA.* 77:3254.
- Wang, K. and Wright, J. 1988. Architecture of the sarcomere matrix of skeletal muscle: Immunoelectron microscopic evidence that suggests a set of parallel inextensible nebulin filaments anchored at the Z-line. *J. Cell Biol.* 107:2199.

- Wang, K., McClure, J., and Tu, A. 1979. Titin a major myofibrillar component of striated muscle. *Proc. Natl. Acad. Sci. USA.* 76:3698.
- Wang, K., Ramirez-Mitchell, R. and Palter, D. 1984b. Titin is an extraordinary long, flexible and slender myofibrillar protein. *Proc. Natl. Acad. Sci. USA.* 81:3685.
- Wang, K., Wright, J. and Ramirez-Mitchell, R. 1984a. Titin: Possible candidate as components of putative longitudinal filaments in striated muscle. *J. Cell Biol.* 97:258a.
- Warner, K.F. 1928. Progress report of the mechanical test for tenderness of meat. *Proceeding of the American Society of Animal Production.* p. 114.
- Wheeler, T.L., Crouse, J.D. and Koohmaraie, M. 1992. The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. *J. Anim. Sci.* 70:3451.
- Wheeler, T.L., Koohmaraie, M. and Crouse, J.D. 1991. Effects of calcium chloride injection and hot boning on the tenderness of round muscles. *J. Anim. Sci.* 69:4871.
- Wheeler, T.L., Koohmaraie, M., Lansdell, J.L., Siragusa, G.R. and Miller, M.F. 1993. Effects of postmortem injection time, injection level, and concentration of calcium chloride on beef quality traits. *J. Anim. Sci.* 71:2965.
- Whipple, G. and Koohmaraie, M. 1993. Calcium chloride marination effects on beef steak tenderness and calpain proteolytic activity. *Meat Sci.* 33:265.
- Whiting, A., Wardale, J. and Trinick, J. 1989. Does titin regulate the length of muscle thick filaments. *J. Mol. Biol.* 205:263.
- Whiting, R.C. and Miller, A.J. 1984. Evaluation of a food processor for making model meat emulsions. *J. Food Sci.* 49:1222.

- Wierbicki, E., Cahill, V.R. and Deatherage, F.E. 1957a. Effects of added sodium chloride, magnesium chloride, calcium chloride and citric acid on meat shrinkage at 70°C and of added sodium chloride on drip losses after freezing and thawing. *Food Tech.* 11:74.
- Wierbicki, E., Konkle, L.E., Klosterman, E.W. and Deatherage, F.E. 1956. Postmortem changes in meat and their possible relations to tenderness together with some comparisons of meat from heifers, bulls, steers, and diethylstilbestrol treated bulls and steers. *Food Tech.* 10:80.
- Wierbicki, E., Kunkle, L.E. and Deatherage, F.E. 1957b. Changes in the water holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifuged method for measuring shrinkage. *Food Tech.* 11:69.
- Winger, R.J. and Fennema, O. 1976. Tenderness and water holding properties of beef muscle as influenced by freezing and subsequent storage at -3°C or 15°C. *J. Food Sci.* 41:1433.
- Winkler, G.A. 1939. Tenderness of meat. I. A recording apparatus for its estimation and relation between pH and tenderness. *Canadian Journal of Research.* 17:18.
- Yamamoto, K. and Moos, C. 1983. The C-proteins of rabbit red, white, and cardiac muscles. *J. Biol. Chem.* 258:8395.
- Young, G.O., Graafhuis, A.E. and Davey, L.C. 1980. Post mortem changes in cytoskeletal proteins of muscle. *Meat Sci.* 5:41.
- Young, L.L. and Lyon, B.B. 1986. Effect of sodium tripolyphosphate in the presence and absence of calcium chloride on water retention properties and shear resistance of chicken breast meat. *Poultry Sci.* 65:898.
- Zeece, M.G., Robson, R.M., Lusby, M.L. and Parrish, F.C., Jr. 1986. Effect of calcium activated protease (CAP) on bovine myofibrils under differed conditions of pH and temperature. *J. Food Sci.* 51:707.



Zot, A.S. and Potter, J.D. 1985. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. *Ann. Rev. Biophys. Chem.* 16:535.

## APPENDIX

Name \_\_\_\_\_

Date \_\_\_\_\_

## SENSORY ANALYSIS OF BEEF

**INSTRUCTIONS:** Place a vertical mark across the horizontal line according to the intensity of the attribute you are testing. Please put the number of the sample above the vertical line when you make it.

**Juiciness:** The sensation of free fluids released from the meat during th first few chews.

very dry \_\_\_\_\_ very juicy

**Softness to tooth pressure:** Measure of how easily the sample compresses between the molars

very hard \_\_\_\_\_ very soft

**Fiber fragmentation:** Measure of how easily the sample breaks down into smaller pieces

very difficult \_\_\_\_\_ very easy

**Bitter:** Degree of bitterness, if any, in each sample

none \_\_\_\_\_ very bitter

**Saltiness:** Degree of saltiness, if any, in each sample

none \_\_\_\_\_ very salty

**COMMENTS:**